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REVIEW ARTICLE

Mechanisms of Hsp90 regulation

Chrisostomos Prodromou*¹

*Genome Damage and Stability Centre, Science Park Road, Falmer, Brighton, East Sussex BN1 9RQ, U.K.

Heat shock protein 90 (Hsp90) is a molecular chaperone that is involved in the activation of disparate client proteins. This implicates Hsp90 in diverse biological processes that require a variety of co-ordinated regulatory mechanisms to control its activity. Perhaps the most important regulator is heat shock factor 1 (HSF1), which is primarily responsible for upregulating Hsp90 by binding heat shock elements (HSEs) within Hsp90 promoters. HSF1 is itself subject to a variety of regulatory processes and can directly respond to stress. HSF1 also interacts with a variety of transcriptional factors that help integrate biological signals, which in turn regulate Hsp90 appropriately. Because of the

diverse clientele of Hsp90 a whole variety of co-chaperones also regulate its activity and some are directly responsible for delivery of client protein. Consequently, co-chaperones themselves, like Hsp90, are also subject to regulatory mechanisms such as post translational modification. This review, looks at the many different levels by which Hsp90 activity is ultimately regulated.

Key words: chaperones, co-chaperones, heat-shock response, HSF1, Hsp90, post-translational modification.

INTRODUCTION

Hsp90 (heat-shock protein 90) accounts for 1–2 % of the cellular protein and rises to 4–6 % in stressed cells [1–4]. Levels of Hsp90 in cells are dependent on the master HSR (heat-shock response) regulator HSF1 (heat-shock factor 1), which is subject to a complex set of regulatory processes. Additionally, Hsp90 is regulated by other mechanisms that have an impact on its transcription, and is subject to post-translational modification and regulation by co-chaperones. Human cells contain a constitutively expressed Hsp90 β (*HSP90AB1*) and a heat-inducible Hsp90 α (*HSP90AA1*) [5], that were separated ~500 million years ago, but still maintain 86 % amino acid sequence identity [6]. Despite high conservation the proteins display different functions [7]. Interestingly, Hsp90 α is not essential in mammals, whereas Hsp90 β is, suggesting that Hsp90 β is involved in processes that maintain viability, whereas Hsp90 α is involved in more adaptive roles [8,9]. Hsp90 is responsible for the maturation of key signalling proteins including regulatory kinases [10–12], steroid hormone receptors [13] and transcription factors [14]. Hsp90 has been implicated in the assembly and disassembly of protein complexes [15] and can suppress phenotypic variation [16–19]. Collectively, Hsp90 α and Hsp90 β interact with approximately 10 % of the eukaryotic proteome [20], representing ~2000 proteins [21], of which, to date, ~725 experimentally determined interactions have been confirmed by direct protein–protein interaction experiments. This implicates Hsp90 in diverse biological processes [3] that necessitate a wide range of mechanisms to regulate its function. The present review examines the major mechanisms, from HSF1 to co-chaperones, which regulate cytoplasmic Hsp90s.

STRUCTURE OF Hsp90

The structure and chaperone cycle of Hsp90 has been extensively reviewed elsewhere [22,23] and, consequently, only a basic description is provided in the present review. Hsp90 consists of three domains: an N-terminal dimerization domain, responsible for binding ATP, which is connected to a middle domain via an unstructured charged linker, and the C-terminal domain, which is responsible for the inherent dimerization of the protein, while the N-terminal domains undergo transient dimerization by binding ATP [24] (Figure 1). Binding of ATP promotes the movement of a lid segment within each N-terminal domain that locates over the bound ATP [25]. The movement of the lids exposes surface residues that are subsequently involved in transient dimerization of the N-terminal domains of Hsp90 (Figures 1B and 1C). ATPase activity of Hsp90 is achieved when the middle domain catalytic loop of Hsp90 moves to an open active state [26] (Figure 2). This loop possesses a conserved arginine residue (Arg³⁸⁰ in yeast), which interacts with the γ -phosphate of ATP, and thus promotes ATP hydrolysis by Hsp90. The active conformation of the catalytic loop is modulated by the binding of the co-chaperone Aha1, which consequently stimulates the ATPase activity of Hsp90 [27]. The conformational changes, including lid closure and modulation of the catalytic loop, represent the rate-limiting step of the chaperone cycle of Hsp90 (Figure 3). Currently, the molecular detail by which the Hsp90 chaperone cycle brings about the activation and maturation of client proteins remains elusive.

ACTIVATION OF HSF1 AND THE HEAT-SHOCK RESPONSE

HSF proteins are responsible for regulating the HSR [28–31], which is induced by a variety of stimuli including elevated

Abbreviations: AhR, aryl hydrocarbon receptor; AMP-PNP, adenosine 5'-[β , γ -imido]triphosphate; Cdk4, cyclin-dependent kinase 4; CHIP, C-terminus of the Hsc (heat-shock cognate) 70-interacting protein; CS, CHORD and Sgt1; CTA, C-terminal transactivation; DBD, DNA-binding domain; eNOS, endothelial nitric oxide synthase; FKBP, FK506-binding protein; HDAC, histone deacetylase; HOP, Hsp70/Hsp90-organizing protein; HSE, heat-shock element; HSF1, heat-shock factor 1; Hsp, heat-shock protein; HSR, heat-shock response; IKK, inhibitor of NF- κ B kinase; IL, interleukin; NF-IL6, nuclear factor for IL-6; NF- κ B, nuclear factor κ B; NTA, N-terminal transactivation; PP5, protein phosphatase 5; RD, regulatory domain; SP1, specificity protein 1; STAT, signal transducer and activator of transcription; Strap, stress-responsive activator of p300; TPR, tetratricopeptide repeat; UPE, upstream promoter element.

¹ email chris.prodromou@sussex.ac.uk

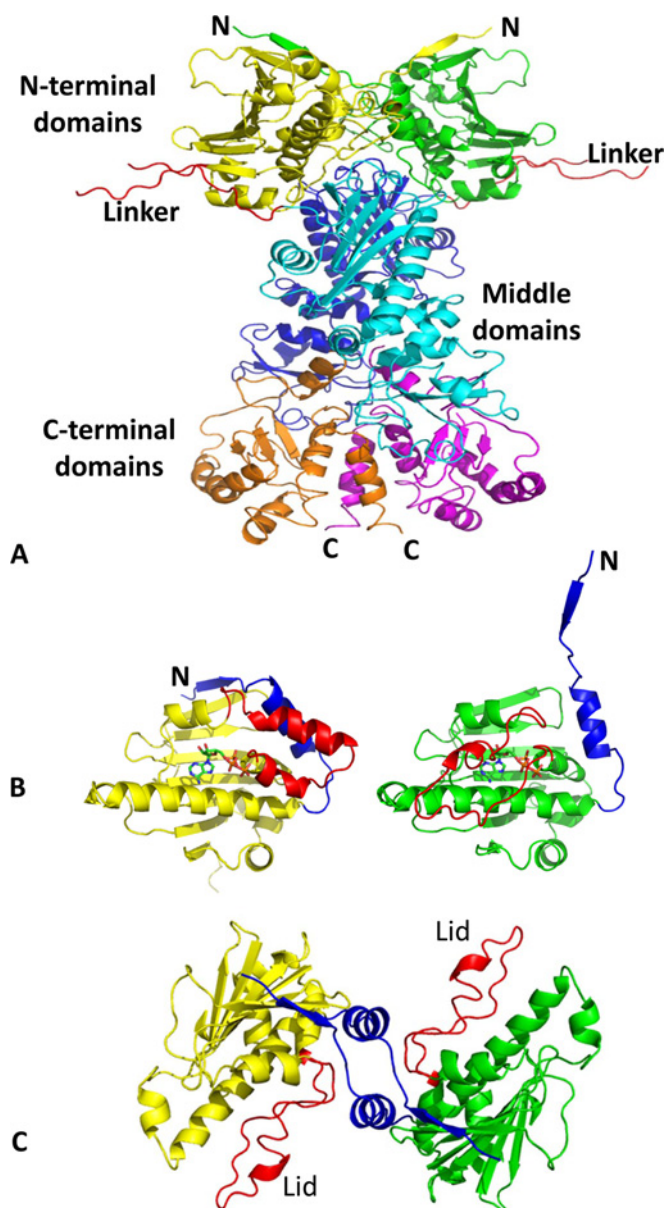


Figure 1 Structure and conformational change in Hsp90

(A) The Hsp90 dimer in a closed conformation involving transient dimerization of the N-terminal domains. N-terminal domains, yellow and green; middle domains, blue and cyan; C-terminal domains, orange and magenta; charged linker, red. (B) Conformation of the lid and N-terminal segment of the N-terminal domains of Hsp90 in the open undimerized state (left-hand panel, yellow) and the closed dimerized state (right-hand panel, green). Lids, red; N-terminal segment, blue. (C) The closed transient N-terminal dimerized state of Hsp90 (yellow and green). Lids, red; N-terminal segment, blue. N-terminal dimerization involves movement of the N-terminal segments of the N-terminal domains and association with the closed lid segments and with the neighbouring N-terminal domains.

temperature, bacterial or viral infection and oxidative stress [32]. As a master regulator of the HSR and a client protein of Hsp90, understanding HSF1 function is central to understanding the regulation of Hsp90.

All HSF proteins consist of a DBD (DNA-binding domain), a trimerization domain consisting of three leucine zipper repeats (HR-A/B), an HR-C region, which negatively regulates the HR-A/B domain, and a CTA (C-terminal transactivation) domain, which is negatively controlled by the central RD (regulatory domain) (reviewed in [30,33–35]) (Figure 4A). Yeast also

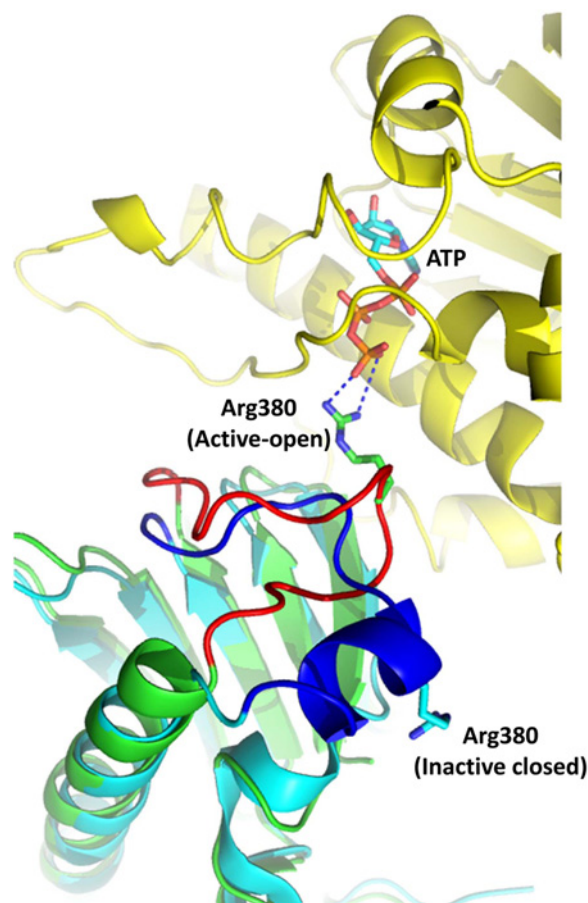


Figure 2 Conformation of the catalytic loop of Hsp90

The N-terminal domain of Hsp90 is shown in yellow. The middle domain is represented by two superimposed molecules of Hsp90 (cyan and green), one with a closed inactive catalytic loop (blue) and the other with an open active state (red) that interacts with the bound ATP, which is shown as a stick model. Arg³⁸⁰ of the catalytic loop is either interacting with the ATP (active state) or is held in an inactive state. Broken blue lines represent hydrogen bonds.

possesses a novel NTA (N-terminal transactivation) domain [36]. The DBD of HSFs bind HSEs (heat-shock elements) that consist of a variable number of nGAAn units (reviewed in [37]) and the precise arrangement of these units can promote cooperativity between binding HSF trimers. The typical types of HSE approximate to three or more contiguous 5 bp repeat motifs (i.e. nTTCnnGAAnnTTCn).

The trimerization domain of HSF consists of two heptad repeats (HR-A and HR-B) that form a triple-stranded α -helical coiled coil [38]. In both *Drosophila* and mammalian HSF1 a third heptad repeat (HR-C) is responsible for intramolecular interactions with HR-A and HR-B, which maintain HSF1 in a monomeric state (Figure 4A), but is readily reversed by stress [39–41]. Trimerization is also stimulated by DBD intermolecular aromatic interactions between a tryptophan and a phenylalanine residue [42], and in mammals by two cysteine residues that form a disulfide bond in response to stress [42,43].

The CTA domain is predominantly unfolded, but its α -helical content increases due to elevated temperature resulting in the hyperphosphorylation of the protein. This is required for sustained increases in transcriptional activation when a single HSF trimer is bound to three nGGAn units [44,45], but dispensable when HSF1 trimers bind co-operatively to HSEs consisting of four or more nGAAn units [46–48], where each HSF1 molecule binds

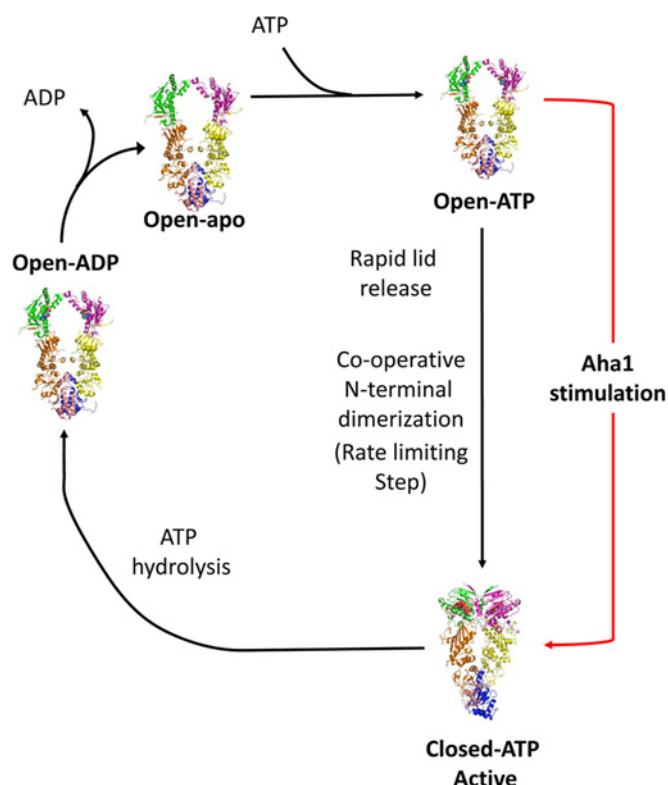


Figure 3 The Hsp90 chaperone cycle

ATP binding triggers transient N-terminal dimerization, through conformational changes in the N-terminal domain of Hsp90 including those of the lid and N-terminal segment, and association with the catalytic loop of the middle domain. These motions act co-operatively to form the catalytically active closed state of Hsp90. Aha1, can accelerate the formation of this closed state by modulating the catalytic loop to an active open state. Once ATP is hydrolysed the N-terminal domains separate, the open inactive state of Hsp90 is formed and ADP is released. Hsp90 is now ready to enter the next cycle.

at least two of the four nGAAn units. In yeast, the NTA domain is unstructured [49] and appears to mediate transient activation of HSF1, suggesting that it behaves as a negative regulator of the CTA domain and that this domain is not wholly sufficient for stress-mediated HSF1 activation [50,51]. The CTA domain drives increasing levels of sustained promoter activity over normal growth temperatures (15–33 °C), but transient activity, directed by the NTA domain, is induced over a higher and narrower temperature range (34.5–39 °C) [52].

During stress, denatured protein levels accumulate [28], which triggers the conversion of the cytoplasmic non-DNA-binding HSF1 into a homotrimer that gains DNA-binding activity. Thus HSF1 is released from its repressed association with Hsp90 [53–55], undergoes homotrimerization [41] and translocation to the nucleus, where it binds HSE [56–58]. However, as yet it is incapable of enhancing transcription and has minimal transactivation competence [59–64]. The next phase involves a series of phosphorylations that transforms the HSF1 trimer into an active transcription factor (reviewed in [34,35]). This leads to a rapid up-regulation of Hsp90 as well as other chaperones and co-chaperones, including Hsp70, Hsp40 and Hsp27 [65].

Under normal conditions, many sites in the RD of HSF1, including Ser²³⁰, Ser³⁰³, Ser³⁰⁷ and Ser³⁶³ are phosphorylated. Phosphorylations at Ser³⁰³, Ser³⁰⁷ and Ser³⁶³ are actually repressive to transcriptional activity, but, significantly, these can be overridden by stress [66,67] and appear to represent a stress-sensitive repressive system [34]. In contrast, phosphorylation at

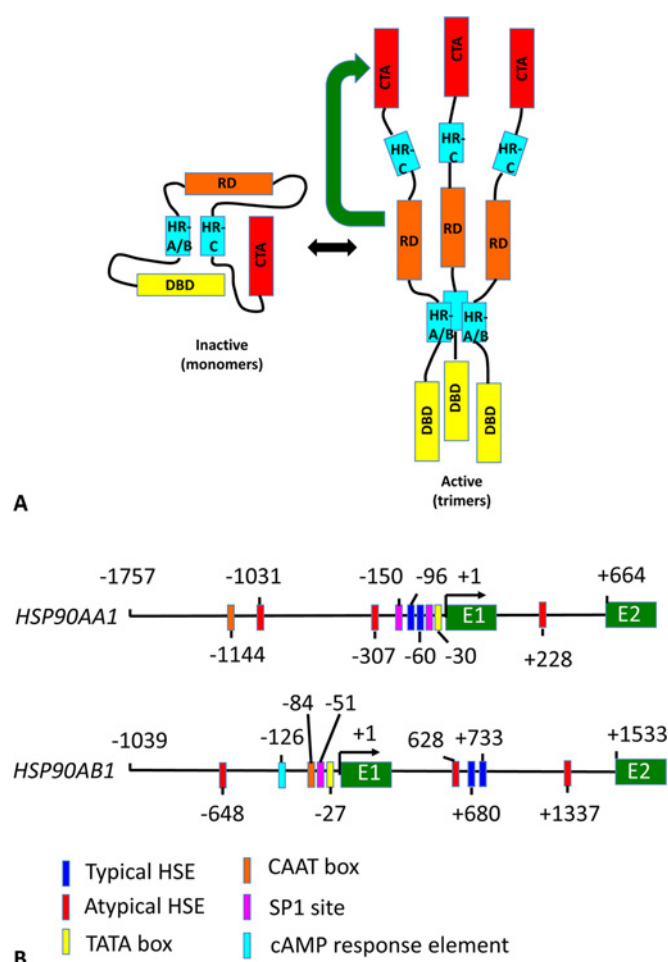


Figure 4 Domain structure of mammalian HSF1 and the promoter and upstream control elements of Hsp90-encoding genes

(A) The domain structure of HSF1. The N-terminal DBD consists of the first 110 amino acids and is followed by the HR-A/B trimerization domain consisting of amino acids 130–203. The RD is encompassed by residues 221–383 and is followed by the HR-C region, residues 384–409, which negatively regulate the HR-A/B trimerization domain. The CTA domain consists of residues 410–529 and is negatively regulated by the RD (green arrow). Activation of HSF1 involves trimerization through the HR-A/B domains. Yeast HSF1 differs in that it also possesses an N-terminal transactivation domain that negatively regulates the RD. (B) Promoter and regulatory regions of human Hsp90-encoding genes. Sequences start from 5' upstream regions, –1757 for *HSP90AA1* and –1039 for *HSP90AB1*, and end at the second exon. The approximate locations of various control elements are indicated and can be identified from the key. E1 and E2 are exons 1 and 2. The UPE (–125 to –37 bp) region of *HSP90AA1* confers a 10-fold up-regulation of the core promoter. The core promoter (–36 to +37 bp) of *HSP90AB1* confers constitutive expression, and the two typical HSE are responsible for maintaining a high level of constitutive expression.

Ser²³⁰ appears to promote transcriptional activity of HSF1 and the basal phosphorylation state at Ser²³⁰ increases upon heat shock. However, the S230A mutation does not wholly repress heat-shock-induced transcriptional activity [68]. Another, very important stimulatory phosphorylation occurs at Ser³²⁶, which promotes the association of the co-activator Daxx, which appears to be an important mediator of HSF1 activation [69]. However, there are numerous kinases that are responsible for HSF1 phosphorylation events (reviewed in [34,35]), and these probably help to integrate signals from different signalling pathways. Although such phosphorylation events appear to be critical for HSF1 activation, recent evidence suggests that it is possible to uncouple the stress-inducible phosphorylation of HSF1 from its

activation, suggesting that the phosphorylation signature of HSF1 alone is not an appropriate marker for HSF1 activity [70]. HSF1 has also been reported to be regulated by SUMOylation [71–74] and acetylation [75–77].

In addition to post-translational modifications, a number of other mechanisms exist that regulate HSF1. The RD of HSF1 carries an intrinsic ability to sense heat stress [78] and Hsp90 might play a role in repressing trimeric HSF1 [53,79]. Furthermore, Hsp70 and Hsp40 appear to be able to inhibit HSF1 transactivating activity, which might occur through the recruitment of a Hsp70-interacting transcriptional co-repressor, CoREST (co-repressor for element-1-silencing transcription factor) [80,81]. A ribonucleoprotein complex consisting of the translation elongation factor eEF1A (eukaryotic elongation factor 1A) and a constitutively expressed non-coding heat-shock RNA-1 RNA has also been reported to act as a HSF1 activator, and *in vitro* can promote the trimerization of HSF1 [82,83]. HSF1 can also associate with the molecular chaperone TriC [84], but whether Hsp90 is chaperoning the assembly of TriC or whether TriC plays a regulatory role in Hsp90 expression remains unknown.

In summary, HSF1 is the master regulator of Hsp90 levels in cells. Consequently, a complex series of regulatory mechanisms have evolved, including transcriptional, HSF1 trimerization, co-operative binding to HSEs, post-translational modification and the ability of HSF1 to detect stress directly. Together these mechanisms integrate a variety of signals that bring about appropriate changes in the level of Hsp90, as well as other heat-shock proteins, in cells.

REGULATION OF Hsp90 GENE EXPRESSION

Once HSF1 is activated it up-regulates the HSR by binding to HSEs upstream of heat-shock genes, such as those encoding Hsp90, Hsp70, Hsp40 and small HSPs. In humans, the complement strand of chromosome 14q32.33 encodes the *HSP90AA1* gene (Hsp90 α), while *HSP90AB1* (Hsp90 β) is located at 6p21. Unusually for molecular chaperones, the human Hsp90-coding genes contain intron sequences and the translational initiation of both genes is located within the beginning of the second exon [85–87]. Within the first intron of *HSP90AB1*, there are two typical HSEs that are responsible for maintaining a high level of constitutive expression [88], in addition to two atypical HSEs (Figure 4B). A third atypical HSE site is located upstream of the transcriptional start site (Figure 4B). In contrast, *HSP90AA1* possesses two typical HSEs immediately upstream of the TATA box, two additional atypical sites further upstream and another atypical site within the first intron [89] (Figure 4B).

The core promoter (–36 to +37 bp) of *HSP90AB1* confers constitutive expression [89]. The promoter of *HSP90AB1* contains a CAAT box, an SP1 site, a TATA box (–27 bp) and a transcriptional start. In contrast, the promoter of *HSP90AA1* contains an SP1 (specificity protein 1) site, a TATA box (–30 bp) and the transcriptional start. The core promoter appears not to contain a CAAT box, although one has been located at –1144 bp (Figure 4B).

The UPE (upstream promoter element) (–125 to –37 bp) region of the *HSP90AA1* gene confers a 10-fold up-regulation of the core promoter. In contrast, the region –1377 to –848 bp has a negative effect on expression, but a further section, –1756 to –1377 bp, provides positive regulation that overcomes this negative effect. The UPE of *HSP90AA1* (–125 to –37 bp) contains an HSE at –96 to –60 bp, in which there is an array of 5 bp HSE motifs (gGAgggTTCTTCcGGAagTTCaaGAggcTTCtgGAAa). The

HSEs derived approximate to gGAgggTTCt, cTTCcgGAA and gTTCaaGAggcTTCtgGAAa, and this has been named the proximal HSE complex and meets the criterion of a typical HSE of at least three adjacent 5 bp motifs [89]. Within the upstream region there are a further five motifs, two located at –1031 to –1022 bp (cGAAaaTTCc) and another that matches the criterion of a typical HSE at –307 to –288 bp (gGGAaccTTCccGAg). Another HSE is found within the first intron at +238 to +247 bp (cTTCagGAAt). During heat shock, induction of *HSP90AA1* is dependent on the coexistence of the distal HSE at –1031 to –1022 bp and the proximal HSE complex [89].

In comparison, the *HSP90AB1* gene possesses a CRE (cAMP-response element) (–126 bp) and a UPE that contains a CAAT box (–87 to –84 bp), an SP1 site (–51 bp), the TATA box at –27 bp and finally the transcriptional start (+1 bp) (Figure 4B). The *HSP90AB1* gene possesses one upstream atypical HSE at –684 to –634 bp (gGAAacTgCtgGAAa) and four HSEs in the first intron of the gene. Two of these are typical HSEs (gTTCtgGAAGAATTCa at +680 to +695 bp and gTTCtgGAAgcttct at +733 to +747 bp), whereas the other two are atypical (cTTCcaGAtctTTCt at +628 to 642 bp and tGAAtTTCa at +1337 to +1346 bp). The upstream HSE appears not to respond to heat shock, whereas the HSEs within the first intron play a vital role [88]. The intronic HSEs of *HSP90AB1*, relative to the atypical sites, are bound tightly by HSF1, and appear to be the most important for maintaining its high constitutive and heat-shock expression levels. Novel initiation sites within the first intron have also been identified for both genes [88,89].

In addition to the activation by HSF1, Hsp90 β is up-regulated by the IL (interleukin)-6 transcription factors NF-IL6 (nuclear factor for IL-6) and STAT-3 (signal transducer and activator of transcription 3) [90]. Furthermore, IFN- γ (interferon- γ) activation of STAT-1 also up-regulates Hsp90 β [90,91]. The binding sites for STAT-1 and STAT-3 appear to overlap with HSEs of HSF1 [90] (Figure 5), and significantly, the DNA-binding sites of STAT proteins (TCCN_{2–4}GAA) are very similar to those of HSF1 (TCCnnGAAnnTTC) [37,92]. Additionally, STAT-1 and HSF1 can interact with each other and bring about strong transcriptional activation, whereas STAT-3 and HSF1 appear to be unable to interact and therefore antagonize each other, resulting in reduced expression of Hsp90 β [90,91]. This leads to a rather complicated regulatory system where STAT-1 and STAT-3 activation leads to activation of *HSP90AB1* promoters, whereas interplay with HSF1 can modulate expression either up or down. It is likely that STAT-1 and STAT-3 play an important role in regulating Hsp90 β under non-stressful conditions, and their interaction with HSF1 is a means by which they are able to integrate their responses with the stress response [90] (Figure 5). Furthermore, NF-IL6 has a similar, but not identical, DNA-binding consensus sequence (TTnnGnAAT) [93,94], but the significance of this, if any, is unknown.

The expression of human Hsp90 is augmented by Strap (stress-responsive activator of p300) [95], a transcription cofactor responsible for the control of the DNA damage response through a mechanism involving regulation of p53 activity [96,97] (Figure 5). Strap was reported as a heat-shock-inducible protein that forms a chromatin-associated complex with HSF1, and the co-activator p300, which has a histone acetylase activity and is required for activation by certain transcription factors [98]. It is thought that the ability to up-regulate Hsp90 expression might involve chromatin acetylation.

The *HSP90AA1* gene is under NF- κ B (nuclear factor κ B) regulatory control (Figure 5). The NF- κ B family of transcription factors regulate the expression of a large variety of genes involved in a number of cellular processes such as inflammation, immune

Table 1 Post-translational modifications occurring in yeast and human Hsp90 and their effects on the Hsp90 chaperone cycle and interactions with client proteins and co-chaperones

yHsp90, yeast Hsp90.

Post-translational modification	Residue	Comment
Phosphorylation	Hsp90 α Thr ⁵ and Thr ⁷	In response to DNA damage
	yHsp90 Thr ²²	Reduces interaction with Aha1 in yeast
	yHsp90 Thr ²²	Affects more than a single class of client protein maturation
	Hsp90 α Thr ³⁶	Promotes the dissociation of Cdc37 ^{p50}
	Hsp90 α Tyr ¹⁹⁷	May be required for ligand-independent epidermal growth factor receptor degradation
Acetylation	Hsp90 α Ser ³⁹¹	Determines the differential binding status of HOP and CHIP
	Hsp90 α Thr ⁷²⁵	Dissociation of AhR and destabilization of AhR
	Hsp90 α Ser ²³¹	
	Hsp90 β Ser ²²⁶ and Ser ²⁵⁵	
	Hsp90 α Lys ⁶⁹ , Lys ¹⁰⁰ , Lys ²⁹² , Lys ³²⁷ , Lys ⁴⁷⁸ , Lys ⁵⁴⁶ and Lys ⁵⁵⁸	Glutamine mutants show decreased binding of nucleotides to Hsp90 (except K292Q)
Nitrosylation	Hsp90 α Lys ¹⁰⁰ , Lys ²⁹² , Lys ³²⁷ , Lys ⁴⁷⁸ , Lys ⁵⁴⁶ and Lys ⁵⁵⁸	Glutamine mutants show decreased binding with co-chaperones and, to a lesser extent, Hsp40
	Hsp90 α Lys ⁶⁹ , Lys ¹⁰⁰ , Lys ³²⁷ , Lys ⁴⁷⁸ , Lys ⁵⁴⁶ and Lys ⁵⁵⁸	Glutamine mutants show reduced binding to CHIP, disrupted binding to Hsp70 and c-Raf (except K327Q)
	Hsp90 α Lys ²⁹²	Glutamine mutant shows decreased association with client proteins, including ErbB2, p60 ^{src} , Raf-1, Hif1, mutant p53 and androgen receptor, and with some co-chaperones, including Aha1, CHIP and FKBP52
SUMOylation	Hsp90 α Cys ⁵⁹⁷	Reported to inhibit Hsp90 α ATPase activity
	yHsp90 Lys ¹⁷⁸ , Hsp90 α Lys ¹⁹¹	Facilitates the association of Aha1 with Hsp90

response, cell growth and development, and is activated as a response to a variety of signals, including cytokines, pathogens, injuries and other stressful conditions [99–106]. A single NF- κ B putative consensus sequence (GGTAGTTCCA) was identified in the 5'-flanking region of the *HSP90AA1* promoter (but not in *HSP90AB1*) [5]. Binding of NF- κ B to this site appears to up-regulate the expression of Hsp90 α . Evidence suggests that HSP90 α is required not only for the biosynthesis of the IKK (inhibitor of NF- κ B kinase) [107], but also for the constitutive and inducible expression of IKK and NF- κ B [107–111]. Thus NF- κ B activity influences *HSP90AA1* gene expression, but reciprocal interactions between the activities of HSP90 α and NF- κ B are likely to constitute a regulatory loop that can influence cell survival and response to stressful agents.

In summary, the core promoter and the two typical HSEs in intron 1 are responsible for maintaining high constitutive expression of Hsp90 β . The intronic HSEs also ensure that *HSP90AB1* is able to respond to heat shock. In contrast, the UPE, in which the proximal HSE complex is situated, confers a 10-fold up-regulation of the core promoter of *HSP90AA1*. The region at –1377 to –848 can promote a negative effect on expression that is overcome by another upstream region at –1756 to –1377. However, during heat-shock induction, the distal HSE at –1031 to –1022 bp together with the proximal HSE complex is responsible for the up-regulation of Hsp90 α . Finally, a variety of other transcriptional regulators are used to integrate diverse cellular signals with the HSR.

POST-TRANSLATIONAL REGULATION OF Hsp90

Because Hsp90 is involved in diverse cellular processes, it is perhaps not surprising that a vast array of post-translational modifications exist for both Hsp90 α and Hsp90 β that regulate their chaperone cycle. These include phosphorylation, acetylation, SUMOylation, methylation, ubiquitylation and S-nitrosylation and have been extensively reviewed in [7,112]. Post-translational modifications discussed in the present review

are shown in Figure 6 in the context of the yeast protein and their effects summarized in Table 1. Although most of these modifications are common to both Hsp90 α and Hsp90 β , others, such as phosphorylation at Thr⁵ and Thr⁷ of Hsp90 α in response to DNA damage [113,114], are specific. This offers a window into understanding not only the differential regulation of cytoplasmic Hsp90s, but also the different processes and functions that these proteins play within cells.

Post-translational modifications have been seen to differentially regulate Hsp90 proteins in response to heat shock within different cellular environments [115,116]. Phosphorylation regulates not only Hsp90 activity directly, but also its ability to interact with chaperones, nucleotides and client proteins [113,115,117–120]. For example, phosphorylation of yeast Hsp90 at Thr²² (human Thr³¹) significantly reduces its interaction with the co-chaperone Aha1 [120,121]. Similarly, the phosphorylation of Hsp90 α at Tyr¹⁹⁷ by the Yes kinase promotes the dissociation of another co-chaperone, Cdc37^{p50}, from Hsp90 α [118]. Another phosphorylation that appears to be unique to Hsp90 α occurs at Ser³⁹¹, and may be required for ligand-independent epidermal growth factor receptor degradation probably through a PNCK (pregnancy-up-regulated non-ubiquitous calmodulin kinase)-dependent pathway [122,123]. The phosphorylation of Thr⁷²⁵ of Hsp90 α determines the differential binding status of the HOP (Hsp70/Hsp90-organizing protein) and CHIP [C-terminus of the Hsc (heat-shock cognate) 70-interacting protein] co-chaperones. It appears that phosphorylation prevents the binding of CHIP, but enhances HOP interaction with Hsp90 α [119]. In contrast, other phosphorylations within Hsp90 α and Hsp90 β appear to have effects only on one isoform. For example, the phosphorylation of Thr⁹⁰ appears to signal the translocation of Hsp90 α to the cell surface for secretion [124,125].

A major role played by phosphorylation of Hsp90 must be to differentially regulate Hsp90s activity with structurally diverse client proteins. For example, the phosphorylation of Ser²³¹ (Hsp90 α) or Ser²²⁶ and Ser²⁵⁵ (Hsp90 β) result in the specific dissociation of AhR (aryl hydrocarbon receptor) and destabilization of AhR. In support of this, alanine mutations

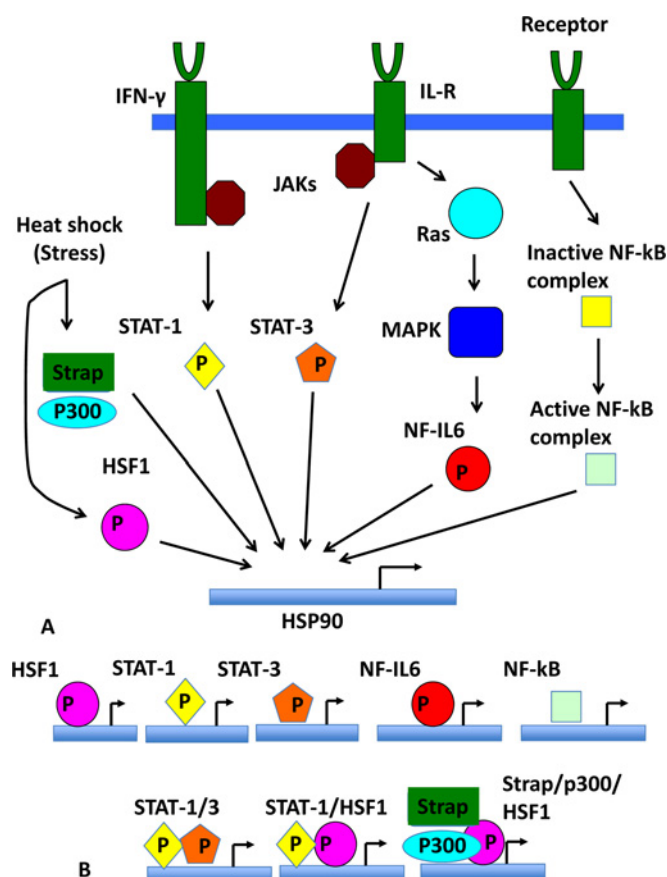


Figure 5 Integration of signalling pathways in the control of Hsp90 expression

(A) Known signalling pathways that affect Hsp90 expression. The co-activator Daxx is not shown in the scheme, but is known to promote the activation of HSF1 [69]. (B) Possible binding scenario for transcriptional activators and cofactors that regulate *Hsp90* transcription. IFN- γ , interferon- γ ; IL-R, interleukin receptor; JAK, Janus kinase; MAPK, mitogen-activated protein kinase.

at these position up-regulated AhR and its association with Hsp90 [126]. In contrast with phosphorylations that differentially regulate Hsp90, others such as Thr²² in yeast Hsp90 and Thr³⁶ in Hsp90 α , appear to affect more than a single class of client protein as was evident using T36A and T36E Hsp90 mutants [120,121]. The kinases that carry out such phosphorylations have been reviewed in [112], and include double-stranded DNA protein kinase, B-raf, Akt, c-Src, protein kinase A, Swe^{Wee1} and casein kinase 2. Although much work has been carried out on determining the effects of Hsp90 phosphorylation, the phosphatases that act on Hsp90 to regulate phosphorylation are not well characterized, although yeast Ppt1 [PP5 (protein phosphatase 5) in humans], positively regulates Hsp90 activity through dephosphorylation [120,125,127].

Hsp90 is also subject to acetylation by p300, whereas deacetylation occurs by a variety of HDACs (histone deacetylases) including HDAC1, HDAC6 and HDAC10 [128–132]. In one study seven acetylated lysine residues were identified in Hsp90 α : Lys⁶⁹, Lys¹⁰⁰, Lys²⁹², Lys³²⁷, Lys⁴⁷⁸, Lys⁵⁴⁶ and Lys⁵⁵⁸ [133]. Using glutamine as an acetylation mimetic, all of the mutants showed decreased binding for nucleotide, except K292Q, which displayed increased binding. The acetylation-mimetic mutants of Lys¹⁰⁰, Lys²⁹², Lys³²⁷, Lys⁴⁷⁸, Lys⁵⁴⁶ and Lys⁵⁵⁸ also displayed decreased binding with co-chaperones and, to a lesser extent, Hsp40. In

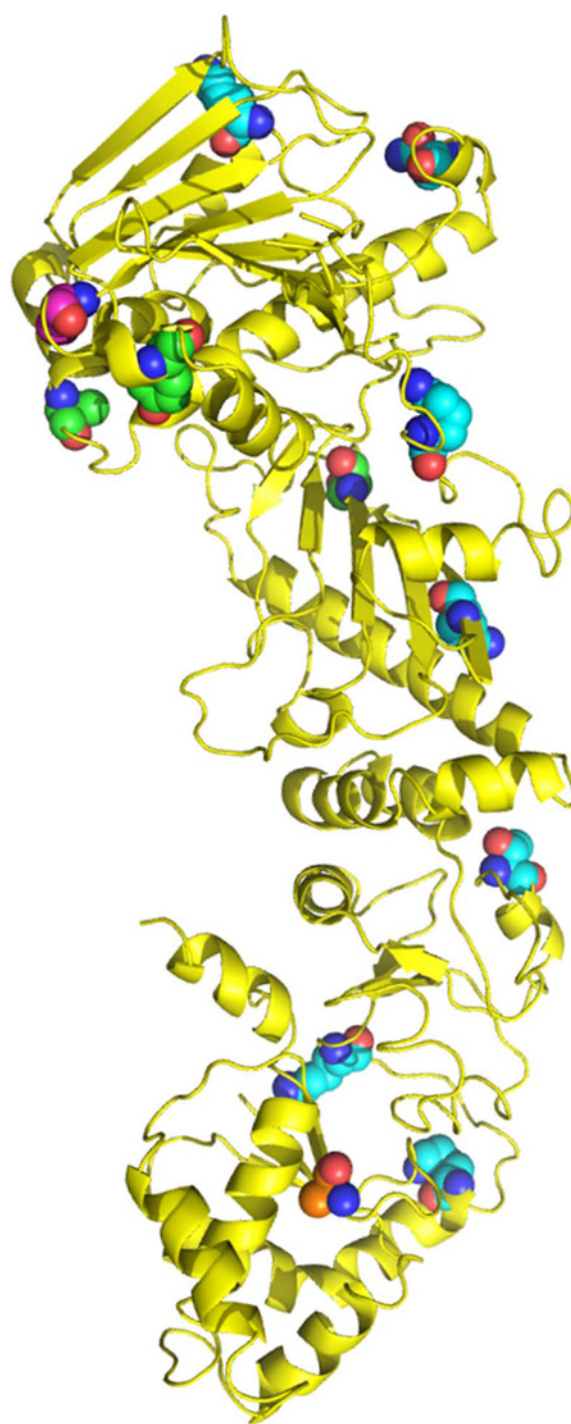


Figure 6 Post-translational modification of Hsp90

A single monomer of yeast Hsp90 (yellow) is shown in cartoon format. Amino acid residues from yeast and human Hsp90 that are post-translationally modified are shown as spheres and mapped to the correct location on yeast Hsp90. Modified amino acid residues for Hsp90 α Ser⁵, Ser⁷ and Ser²³⁴ and Thr⁷²⁵ and Hsp90 β Ser²⁵⁵ that are not represented in the yeast structure are omitted. Green spheres, amino acids that are phosphorylated; cyan spheres, amino acids that are acetylated; magenta spheres, amino acids that are SUMOylated; gold spheres, amino acids that are nitrosylated.

contrast, the glutamine mutants at Lys⁶⁹, Lys¹⁰⁰, Lys³²⁷, Lys⁴⁷⁸, Lys⁵⁴⁶ and Lys⁵⁵⁸ showed reduced binding to CHIP, whereas all of the acetylation mutants, except K327Q, disrupted binding to Hsp70 and with c-Raf. The acetylated mimetic K292Q

was also reported to show a decreased association with client proteins, including ErbB2, p60^{v-src}, Raf-1, Hif1, mutant p53, and androgen receptor and with some co-chaperones, including Aha1, CHIP and FKBP52 (FK506-binding protein 52) [134]. Clearly, these results suggest that acetylation has a major impact on the regulation of human Hsp90.

Other modifications that have been reported include S-nitrosylation, ubiquitylation and SUMOylation. S-nitrosylation at Cys⁵⁹⁷ by nitric oxide (NO) was reported to inhibit Hsp90 α ATPase activity and may represent a negative-feedback loop reducing the activation of eNOS (endothelial nitric oxide synthase), which is Hsp90-dependent [135]. The mechanism by which S-nitrosylation appears to affect Hsp90 might be through an allosteric mechanism bringing about the inhibition of its ATPase activity [136]. An increase in the ubiquitylation of human Hsp90 was shown to inhibit its function and cause a dissociation of client protein, including p53, Cdk4 (cyclin-dependent kinase 4) and Plk (Polo-like kinase 1), Akt1 and eNOS, which were subsequently degraded by the proteasome [137,138]. Swe1 phosphorylation of human Hsp90 signals its ubiquitylation and degradation by the proteasome, but the underlying detailed mechanism is unknown [139].

As with ubiquitylation of Hsp90, oxidative stress, which results in the direct oxidation of cysteine residues, also leads to client protein degradation, including Cdk4, cyclin D1, Raf-1, Akt and mutant p53 [140]. Finally, the asymmetric SUMOylation of the N-terminal domain of Hsp90 [Lys¹⁷⁸ (yeast) and Lys¹⁹¹ (Hsp90 α)] appears to facilitate the association of Aha1 with Hsp90 [141].

In summary, post-translational modifications of Hsp90 offer a means by which the chaperone cycle can be modulated. In particular, it aids the activation of a structurally diverse clientele by Hsp90 by allowing a means by which specific Hsp90s can be differentially regulated. Consequently, a vast array of post-translational modifications are involved in regulating Hsp90, but, for the most part, the regulatory mechanisms involved in these processes are still poorly understood.

THE ATPase ACTIVITY OF Hsp90 AND REGULATION BY CO-CHAPERONES

Determining the exact mechanism by which Hsp90 hydrolyses ATP [24] is critical to understanding how client protein and co-chaperones regulate this activity. The crystal structure of the yeast N-terminal domain of Hsp90 in complex with AMP-PNP (adenosine 5'-[β , γ -imido]triphosphate), a non-hydrolysable analogue of ATP, provided the first direct evidence that Hsp90 was an ATPase [142–145]. Currently all Hsp90s are considered to be ATPases [146–149]. The structure of full-length yeast Hsp90 in complex with Sba1 provided the mechanistic detail by which the catalytically active state of Hsp90 forms, following the binding of ATP [22,25,150].

Rather than ATP hydrolysis [151], the rate-limiting step appears to be conformational change [24], which is now supported by recent kinetic analyses and structural data [25,152,153]. Previously, multi-exponential kinetics using FRET were interpreted as the formation of discrete conformational intermediates in the catalytic cycle [22,25,150,153–155]. However, recent work using yeast Hsp90 and 1-nm fluorescence probes based on photoinduced electron transfer, suggest a mechanism where closure of the lids, β -strand exchange and association of N- and M-domains share similar kinetics, and that these conformational changes act co-operatively to produce the catalytically active state ([156], and Andrea Schulze, Gerti Beliu, Dominic A Helmerich, Jonathan Schubert, Laurence H Pearl,

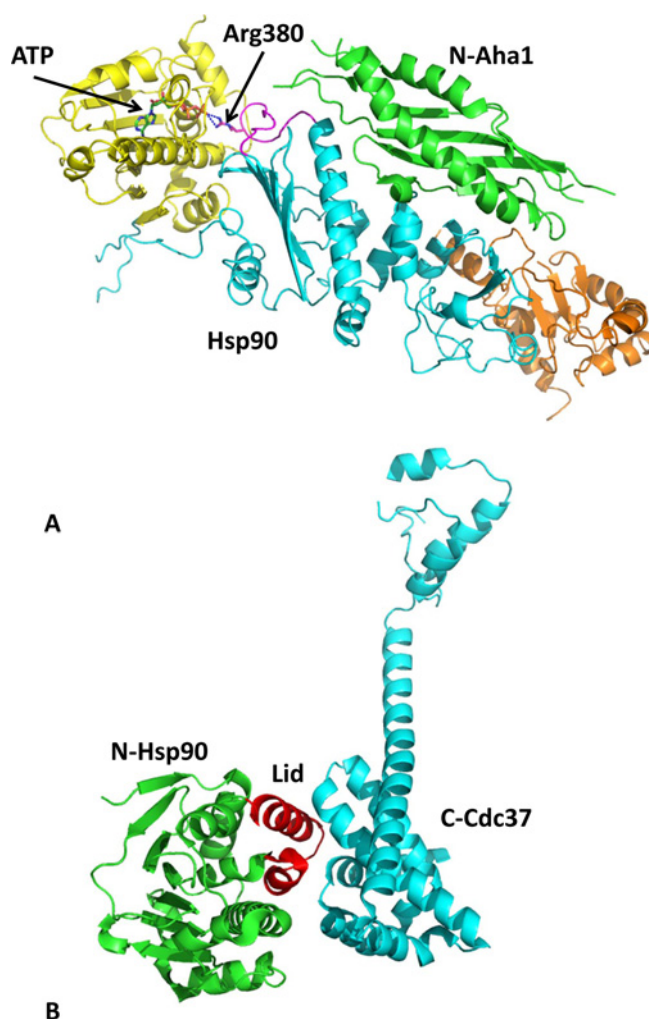


Figure 7 Structure of Hsp90–Aha1 and Hsp90–Cdc37^{p50} co-chaperone complexes

(A) Structure of the Hsp90–Aha1 complex by superimposition of the middle domain of Hsp90 (cyan) in complex with Aha1 (green) on to the full-length structure of Hsp90 (N-terminus, yellow; C-terminus, gold). The binding of Aha1 causes the catalytic loop of Hsp90 (magenta) to move to its open state and allows Arg³⁸⁰ to interact with the γ -phosphate of ATP (green stick representation). Broken blue lines represent hydrogen bonds. (B) Structure of the N-terminal domain of Hsp90 (green) in complex with the C-terminal domain of Cdc37^{p50} (cyan). Cdc37^{p50} binds to the lid segment (red) of the N-terminal domains of Hsp90, preventing them from conformational movements that are required for the formation of the catalytically active state through N-terminal dimerization.

Chrisostomos Prodromou and Hannes Neuweiler, unpublished work) (Figure 3). Thus binding of ATP rapidly releases the lid from its well-ordered open state to a dynamic intermediate. Full closure of the lid over the nucleotide-binding pocket occurs relatively slowly, but significantly in a co-operative manner with inter- and intra-subunit associations between the NTD and M-domains, and reciprocal exchange of the N-terminal β -strands. Furthermore, it appears that Aha1 remodels the catalytic loop in the M-domain of yeast Hsp90 into a conformation favouring engagement with ATP (Figure 7A), by stabilizing N/M-domain interactions [25,157], and by acting directly on the lid to accelerate closure. It was concluded that these conformational changes acting in concert limit the overall rate constant of ATP hydrolysis. With this in mind, we can now look at the effect of co-chaperones on the Hsp90 ATPase activity in a new light.

The detailed biochemical and structural mechanistic effects of co-chaperones on the ATPase activity of Hsp90 has been reviewed in detail [22]. Consequently, I will only cover the mechanism of Hsp90 regulation in the light of the co-operative mechanism for N-terminal dimerization. One of the major roles played by co-chaperones, such as HOP/Sti1, Cdc37^{p50} and Sgt1, is the delivery of client protein to Hsp90 (see [22,23] for reviews). HOP, together with Hsp70, is responsible for delivering steroid hormone receptor to human Hsp90. In so doing, HOP, as well as the yeast orthologue Sti1, inhibits the ATPase activity of Hsp90 [158,159]. This probably represents a critical step that allows steroid hormone receptor to engage with human Hsp90. The primary binding site for HOP/Sti1 on Hsp90 is a highly conserved MEEVD motif that occurs at the extreme C-terminus of Hsp90 [160]. Additional contacts to the C-terminal, middle- and N-terminal domains of Hsp90 have been revealed by biochemical and structural studies with both the yeast and human protein [158,161,162]. Multiple interaction sites between HOP and human Hsp90 are supported by more recent evidence [163] and also suggests that monomeric HOP can bind to Hsp90 [163]. This supports the previous finding that Sti1 prevents N-terminal dimerization by interacting with the first 24 amino acid residues of yeast Hsp90, in addition to the conserved MEEVD motif of Hsp90 [162]. In the light of a mechanism involving co-operative N-terminal dimerization, the ability of Sti1/HOP to interact with the first 24 N-terminal amino acid residues could compromise this process (Figure 8). Such a mechanism is compatible with the observation that HOP, as a monomer, can inhibit Hsp90 ATPase activity, while simultaneously allowing access for the binding of immunophilins, which would promote progression of the chaperone cycle [159].

Cdc37^{p50} is involved in delivering client protein kinases to the Hsp90 complex, and, in a similar way to HOP and Sti1, it inhibits the ATPase activity of Hsp90 [164]. Thus Cdc37^{p50} binds between the N-terminal domains of Hsp90, interacting directly with the lids [165]. This prevents motions in the lids that would otherwise promote co-operative N-terminal dimerization (Figures 7B and 8). Exactly how Cdc37^{p50} exits the complex allowing progression of the chaperone cycle is currently unknown. However, the co-operative mechanism for N-terminal dimerization perhaps offers an explanation how this might occur (Andrea Schulze, Gerti Beliu, Dominic A Helmerich, Jonathan Schubert, Laurence H Pearl, Chrisostomos Prodromou and Hannes Neuweiler, unpublished work). The results of this study suggested that the co-chaperone Aha1 enhances the ATPase activity of Hsp90 by releasing the lid early in the catalytic cycle. It is therefore conceivable that Aha1 displaces Cdc37^{p50} by modulating the catalytic loop of the middle domain and by promoting the movement of the lids towards a closed state favouring N-terminal dimerization (Figure 8).

Another co-chaperone that appears to be involved in client protein loading, by acting as hub for the formation of a variety of Hsp90 complexes, is Sgt1 [166–174]. Together with another co-chaperone, Rar1, Sgt1 plays a central role in the innate immunity response in plants. Sgt1 associates with the CBF3 kinetochore complex, with SCF E3 ubiquitin ligases, with plant R proteins and the related animal Nod-like receptors [175–182]. Sgt1 consists of three domains: an N-terminal TPR (tetratricopeptide repeat) domain, a middle CS (CHORD and Sgt1) domain, and a C-terminal SGS (Sgt1-specific) domain. The N-terminal TPR domain of Sgt1 is similar to other TPR domains that bind the conserved MEEVD motif of Hsp90. Surprisingly, however, the TPR domain of Sgt1 interacts directly with Skp1 [182], whereas the middle CS domain binds Hsp90 [182]. Structural details showing the CS domain interacting with the N-terminal domain of Hsp90 have been published [169]. Although the CS domain of Sgt1 is similar to that of Sba1/p23,

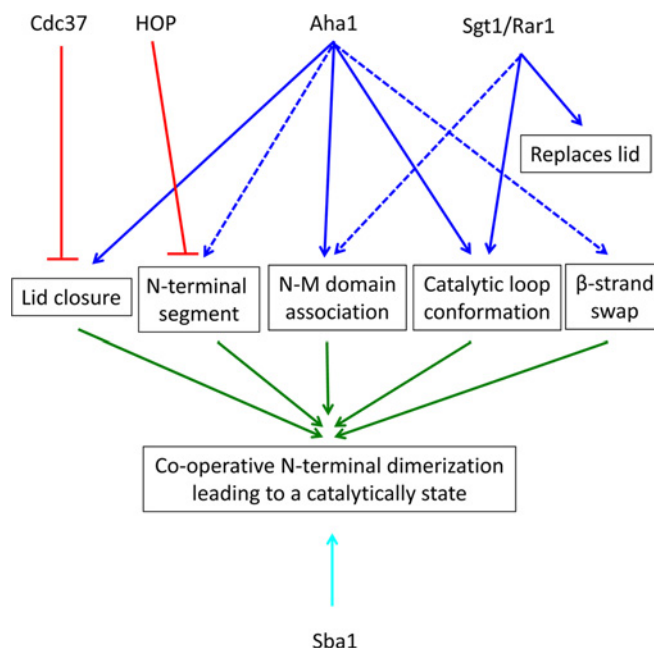


Figure 8 Co-chaperone pathways that modulate Hsp90 ATPase activity

Cdc37^{p50} binds to the lids and prevents molecular rearrangement of Hsp90. HOP appears to interact with N-terminal segment of Hsp90 and thus may prevent N-terminal dimerization. Aha1 is able to interact with possibly all of the structural elements that lead to co-operative N-terminal dimerization of Hsp90. Sba1 interacts with the lid and N-terminal domains of Hsp90 and stabilizes Hsp90 in a closed state that displays a lower rate of ATP hydrolysis. Sgt1, together with Rar1, is unusual in that it activates Hsp90 in an open state and leads to a stable ADP-bound complex. Red and blue arrows indicate a mechanism resulting in the inhibition and activation of ATPase activity respectively. Broken blue arrows indicate interactions that might occur. The cyan arrow indicates a means by which the rate of ATPase activity is decreased. The green arrows indicate the co-operative nature of N-terminal dimerization.

unlike this latter co-chaperone, it does not regulate the ATPase activity of Hsp90 [169,182]. Instead, it recruits Rar1, a plant co-chaperone, or Chp1 and melusin in mammals, which, in the case of Rar1, weakly stimulates the Hsp90 ATPase activity [170]. The structure of the CS domain of Sgt1 and the CHORD II domain of Rar1 in complex with the N-terminal domain of Hsp90 has been published [170] (Figure 9A). In contrast with the activation of the ATPase activity by Aha1, the CHORD II domain of Rar1 stimulates the ATPase activity of Hsp90 in its open conformation. This promotes a stable ADP-bound open-state complex (Figure 9A). It appears that the CHORD II domain replaces the ATP lid and simultaneously modulates the middle domain catalytic loop to achieve activation of Hsp90. It is possible that the Rar1–Sgt1 complex might help promote N- to M-domain association of Hsp90, but currently this is unknown. The Hsp90–Sgt1–Rar1 complex perhaps mimics Hsp90's catalytically active state, without N-terminal dimerization taking place. It therefore appears that Sgt1 and Rar1 stabilize the Hsp90 complex [178] by converting it into a long-lived ADP–Rar1–Hsp90–Sgt1 state [170].

Aha1 is the only co-chaperone known to strongly accelerate the ATPase cycle of Hsp90 [149,183]. Structural studies have shown that the N-terminal domain of Aha1 can modulate the middle domain catalytic loop of Hsp90, stabilizing it in an open active state (Figure 7A). Movement of the catalytic loop to its active state is now known to be required for co-operative dimerization by Hsp90 (Andrea Schulze, Gerti Beliu, Dominic A Helmerich, Jonathan Schubert, Laurence H Pearl, Chrisostomos Prodromou and Hannes Neuweiler, unpublished work), which would promote

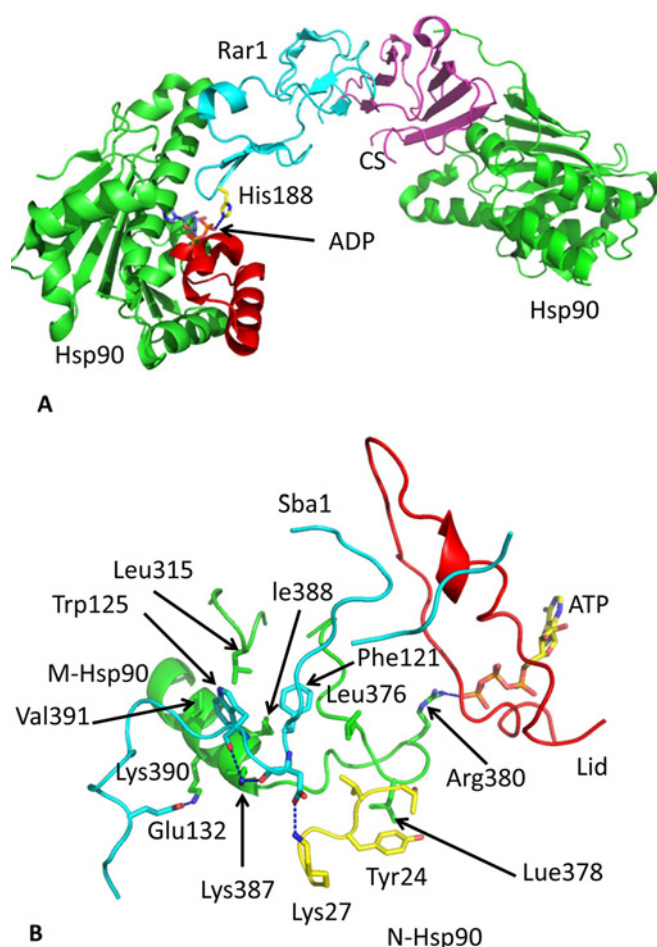


Figure 9 Structure of Hsp90–Rar1–Sgt1 and Hsp90–Rar1–Sba1 co-chaperone complexes

(A) Structure of the N-terminal domains of Hsp90 (green) in complex with the CS domain of Sgt1 (magenta) and the CHORD II domain of Rar1 (cyan). Recruitment of Rar1 into the Hsp90 complex stimulates ATP hydrolysis producing a stable ADP-bound Hsp90 complex. The lid segment is shown in red and bound ADP in blue stick representation. Broken blue lines represent hydrogen bonds. (B) Structure of the closed conformation of Hsp90 in complex with Sba1 (cyan). Hsp90 is represented by the lid (red), the N-terminal segment of the N-terminus (yellow) and a segment of the middle domain (green). The bound ATP is shown as a yellow stick representation. Arg³⁸⁰ is seen to interact with the γ -phosphate of ATP in the catalytically active state of Hsp90. Broken blue lines represent hydrogen bonds.

the ATPase activity of Hsp90. The observation that full-length Aha1 promotes the release of the Hsp90 lids early in the catalytic cycle is consistent with the co-operative nature of N-terminal dimerization. Furthermore, the binding of the C-terminal domain of Aha1 to the N-terminal domains of Hsp90 [184] is supportive of the idea that Aha1 promotes release of the lids early in the chaperone cycle (Andrea Schulze, Gerti Beliu, Dominic A Helmerich, Jonathan Schubert, Laurence H Pearl, Chrisostomos Prodromou and Hannes Neuweiler, unpublished work), and of the observation that full-length Aha1 is required for maximum stimulation of the ATPase activity of Hsp90 [149].

Sba1/p23 shows a higher affinity for the ATP-bound N-terminally dimerized state of Hsp90, rather than the apo or ADP-bound state [150,185]. Since Sba1 binds Hsp90 following the co-operatively driven mechanism of N-terminal dimerization, its role appears to be one of stabilizing the closed state of Hsp90. Sba1/p23, unlike co-chaperones that deliver client proteins to Hsp90 (Sti1, Cdc37^{p50} and Sgt1), therefore acts late in the

Hsp90 chaperone cycle, which Sba1 appears to slow down, rather than totally inhibit [149]. In contrast, a more robust inhibition was reported for the human orthologue p23 [185]. The structure of the full-length yeast Hsp90 in complex with Sba1 and AMP-PNP explains the inhibitory effect of Sba1 binding [25]. The binding of Sba1 to the closed N-terminally dimerized domains of Hsp90 locks the N-terminal domains together, while simultaneously stabilizing the middle domain catalytic loop in an active conformation through a direct interaction (Figure 9B). Thus Sba1 temporally stabilizes the closed N-terminally dimerized state of Hsp90 by slowing its ATPase cycle [149].

In conclusion, the structural variety of Hsp90 clientele necessitates a diverse array of co-chaperones that help deliver clients to Hsp90 and so regulate their activation and maturation. Recent evidence suggests that Hsp90 undergoes N-terminal dimerization involving a co-operatively driven mechanism of structural change. In the light of this, the role played by a variety of Hsp90 co-chaperones can now be reinterpreted and a better understanding of their effect on the Hsp90 cycle is beginning to emerge.

REGULATION OF Hsp90 ACTIVITY THROUGH POST-TRANSLATIONAL MODIFICATION OF CO-CHAPERONES

Post-translational modification of co-chaperones adds further mechanisms by which Hsp90 activity can be regulated. Phosphorylation of Hsp90 co-chaperones has not been studied extensively, but its importance is demonstrated by a number of examples. Ser¹³ of human Cdc37^{p50} is the target for phosphorylation by protein kinase CK2, which appears to be necessary for kinase client chaperoning, including Cdc28^{Cdc2}, Ste11^{RAF}, Kin28, Mps and CK2 itself [186–188]. The phosphorylation appears to favour the formation of a Cdc37^{p50}–Hsp90–kinase complex and dephosphorylation by PP5/Ppt1 appears to weaken this association and might act as a signal for progression of the cycle and release of activated client kinase protein [189].

Another example involving CK2 phosphorylation is that of Sgt1 at Ser³⁶¹, which inhibits the dimerization of the co-chaperone [190]. This in turn influences kinetochore assembly and chromosome segregation in eukaryotes during cell division [190]. Sgt1 acts as an adaptor in several other processes such as the regulation of innate immunity systems in plants and animals and in SCF E3 ubiquitin ligase-directed protein degradation [169]. Whether this phosphorylation has a negative impact on these processes is currently unknown.

CK2 is responsible for the phosphorylation of p23 (also known as cytoplasmic prostaglandin E synthase 3) on Ser¹¹³ and Ser¹¹⁸, and promotes the synthesis of prostaglandin E₂ [191]. p23 is involved in a variety of other client protein complexes including telomerase and steroid hormone receptors [192,193]. The details of how it affects their activation are currently unknown. Ser¹¹³ and Ser¹¹⁸ are not conserved in Sba1, the yeast orthologue of p23. In yeast, the equivalent residues are upstream of Trp¹²⁴, which interacts with the long helix of the middle domain of Hsp90 and is thought to modulate the catalytic loop of this domain. Whether phosphorylation of human Ser¹¹³ and Ser¹¹⁸ affects the ability of p23 to modulate the catalytic loop of Hsp90 is unknown.

Yet another CK2-mediated phosphorylation has been seen in murine mSti1^{HOP} at Thr¹⁸⁹. In contrast, Cdc2 phosphorylates mSti1^{HOP} at Thr¹⁹⁸. It has therefore been suggested that mSti1^{HOP} plays a role in the cell cycle [194]. Finally, CK2 directed phosphorylation of FKBP52 is seen at Thr¹⁴³ and is thought to play a role in steroid hormone activation [186,195]. FKBP phosphorylation at an unspecified site influences the efficiency of adeno-associated virus type-2 transduction [196–198].

For kinases, the progression of the chaperone cycle requires both Cdc37^{p50} and Aha1. The phosphorylation of Cdc37^{p50} at Tyr⁴ and Tyr²⁹⁸ was reported to disrupt client–Cdc37^{p50} association and provided directionality to the cycle [118]. In contrast, phosphorylation of Hsp90 at Tyr¹⁹⁷ by the Yes kinase, was reported to cause dissociation of Cdc37^{p50} from Hsp90 [118], whereas phosphorylation on Tyr³¹³ promotes recruitment of Aha1, both of which further the chaperoning process by stimulating Hsp90 ATPase activity. The phosphorylation of human Aha1 at Tyr²²³ by c-Abl kinase has been reported [199] and appears to promote its interaction with Hsp90. The increased binding of Aha1 is thought to translate into an enhanced activation of Hsp90 ATPase activity, which in turn promotes Hsp90 interaction with kinase clients. In contrast, glucocorticoid receptor and CFTR (cystic fibrosis transmembrane receptor) interactions with Hsp90 were compromised. Unexpectedly, it was reported by the same authors that Tyr²²³ phosphorylation led to ubiquitination and proteasome degradation of Aha1. Finally, Hsp90 α phosphorylation at Tyr⁶²⁷ induces dissociation of the client and remaining co-chaperones that signals completion of the chaperone cycle.

In conclusion, a variety of co-chaperones are required to deliver client protein and to promote the chaperone cycle of Hsp90. The complex nature of the Hsp90 chaperone cycle has allowed co-chaperone regulation of Hsp90 by a variety of mechanisms that involve modulating the co-operative nature of N-terminal dimerization within Hsp90. Ultimately the precise regulatory effect of such co-chaperones is dependent on their post-translational modification and that of Hsp90 itself.

CONCLUDING REMARKS

The cytoplasmic Hsp90 proteins are required for a whole host of biological processes, including adaptation to stress. It is therefore not surprising that the Hsp90 levels in cells is abundant and increases further during stress adaptation. Because of the multitude of tasks carried out by Hsp90, numerous regulatory systems operate to ensure the proper integration and regulation of Hsp90 activity. Most importantly, HSF1 emerges as a master regulator of the HSR, helping integrate a variety of cellular signals into Hsp90 transcriptional control. As such, Hsp90 is a highly regulated protein and is subject to many post-translational modifications as well as being able to sense heat stress directly. The regulation of Hsp90 is controlled by post-translational modifications, and by co-chaperones and client proteins, of which the latter are subject to various regulatory processes themselves. Although much progress has been made in understanding these processes, there remains a significant amount we still do not fully understand. For example, we have recently established some of the post-translational modifications that operate to regulate both Hsp90 and its co-chaperones; however, our knowledge of the processes that control these modifications are still in their infancy. Many of the enzymes, such as kinases, phosphatases, histone acetylases and histone deacetylases remain to be identified. Determining how these modifications are integrated into coherent regulatory systems will not be easy, but is essential if we are to understand the Hsp90 chaperone cycle in the context of the various biological processes that depend on it.

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REFERENCES

- Picard, D. (2002) Heat-shock protein 90, a chaperone for folding and regulation. *Cell Mol Life Sci.* **59**, 1640–1648 [CrossRef PubMed](#)
- Whitesell, L. and Lindquist, S.L. (2005) HSP90 and the chaperoning of cancer. *Nat Rev Cancer.* **5**, 761–772 [CrossRef PubMed](#)
- Taipale, M., Jarosz, D.F. and Lindquist, S. (2010) HSP90 at the hub of protein homeostasis: emerging mechanistic insights. *Nature reviews. Molecular cell biology.* **11**, 515–528 [CrossRef PubMed](#)
- Finka, A. and Goloubinoff, P. (2013) Proteomic data from human cell cultures refine mechanisms of chaperone-mediated protein homeostasis. *Cell Stress Chaperones.* **18**, 591–605 [CrossRef PubMed](#)
- Ammirante, M., Rosati, A., Gentilella, A., Festa, M., Petrella, A., Marzullo, L., Pascale, M., Belisario, M.A., Leone, A. and Turco, M.C. (2008) The activity of hsp90 alpha promoter is regulated by NF-kappa B transcription factors. *Oncogene.* **27**, 1175–1178 [CrossRef PubMed](#)
- Gupta, R.S. (1995) Phylogenetic analysis of the 90 kD heat shock family of protein sequences and an examination of the relationship among animals, plants, and fungi species. *Molecular biology and evolution.* **12**, 1063–1073 [PubMed](#)
- Zuehlke, A.D., Beebe, K., Neckers, L. and Prince, T. (2015) Regulation and function of the human HSP90AA1 gene. *Gene.* **570**, 8–16 [CrossRef PubMed](#)
- Grad, I., Cederroth, C.R., Walicki, J., Grey, C., Barluenga, S., Winssinger, N., De Massy, B., Nef, S. and Picard, D. (2010) The molecular chaperone Hsp90alpha is required for meiotic progression of spermatocytes beyond pachytene in the mouse. *PLoS one.* **5**, e15770 [CrossRef PubMed](#)
- Voss, A.K., Thomas, T. and Gruss, P. (2000) Mice lacking HSP90beta fail to develop a placental labyrinth. *Development.* **127**, 1–11 [PubMed](#)
- Pearl, L.H. (2005) Hsp90 and Cdc37 – a chaperone cancer conspiracy. *Current opinion in genetics & development.* **15**, 55–61 [CrossRef](#)
- Horejsi, Z., Takai, H., Adelman, C.A., Collis, S.J., Flynn, H., Maslen, S., Skehel, J.M., de Lange, T. and Boulton, S.J. (2010) CK2 phospho-dependent binding of R2TP complex to TEL2 is essential for mTOR and SMG1 stability. *Molecular cell.* **39**, 839–850 [CrossRef PubMed](#)
- Takai, H., Xie, Y., de Lange, T. and Pavletich, N.P. (2010) Tel2 structure and function in the Hsp90-dependent maturation of mTOR and ATR complexes. *Genes & development.* **24**, 2019–2030 [CrossRef](#)
- Pratt, W.B., Morishima, Y., Murphy, M. and Harrell, M. (2006) Chaperoning of glucocorticoid receptors. *Handbook of experimental pharmacology* 111–138 [CrossRef](#)
- Pratt, W.B. (1998) The hsp90-based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors. *Proc Soc Exp Biol Med.* **217**, 420–434 [CrossRef PubMed](#)
- Makhnevych, T. and Houry, W.A. (2012) The role of Hsp90 in protein complex assembly. *Biochimica et biophysica acta.* **1823**, 674–682 [CrossRef PubMed](#)
- Rutherford, S.L. and Lindquist, S. (1998) Hsp90 as a capacitor for morphological evolution. *Nature.* **396**, 336–342 [CrossRef PubMed](#)
- Lindquist, S. (2009) Protein folding sculpting evolutionary change. *Cold Spring Harbor symposia on quantitative biology.* **74**, 103–108 [CrossRef PubMed](#)
- Yahara, I. (1999) The role of HSP90 in evolution. *Genes Cells.* **4**, 375–379 [CrossRef PubMed](#)
- Williams, T.A. and Fares, M.A. (2010) The effect of chaperonin buffering on protein evolution. *Genome biology and evolution.* **2**, 609–619 [CrossRef PubMed](#)
- Zhao, R., Davey, M., Hsu, Y.C., Kaplaneck, P., Tong, A., Parsons, A.B., Krogan, N., Cagney, G., Mai, D., Greenblatt, J., Boone, C., Emili, A. and Houry, W.A. (2005) Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone. *Cell.* **120**, 715–727 [CrossRef PubMed](#)
- Echeverria, P.C., Bernthaler, A., Dupuis, P., Mayer, B. and Picard, D. (2011) An interaction network predicted from public data as a discovery tool: application to the Hsp90 molecular chaperone machine. *PLoS one.* **6**, e26044 [CrossRef PubMed](#)
- Prodromou, C. (2012) The 'active life' of Hsp90 complexes. *Biochimica et biophysica acta.* **1823**, 614–623 [CrossRef PubMed](#)
- Prodromou, C. and Morgan, R.M.L. (2016) "Tuning" the ATPase Activity of Hsp90. *Adv Biochem Health D.* **14**, 469–490
- Prodromou, C., Panaretou, B., Chohan, S., Siligardi, G., O'Brien, R., Ladbury, J.E., Roe, S.M., Piper, P.W. and Pearl, L.H. (2000) The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. *The EMBO journal.* **19**, 4383–4392 [CrossRef PubMed](#)
- Ali, M.M., Roe, S.M., Vaughan, C.K., Meyer, P., Panaretou, B., Piper, P.W., Prodromou, C. and Pearl, L.H. (2006) Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex. *Nature.* **440**, 1013–1017 [CrossRef PubMed](#)
- Meyer, P., Prodromou, C., Hu, B., Vaughan, C., Roe, S.M., Panaretou, B., Piper, P.W. and Pearl, L.H. (2003) Structural and functional analysis of the middle segment of hsp90: implications for ATP hydrolysis and client protein and cochaperone interactions. *Molecular cell.* **11**, 647–658 [CrossRef PubMed](#)

- 27 Meyer, P., Prodromou, C., Liao, C., Hu, B., Mark Roe, S., Vaughan, C.K., Vlasic, I., Panaretou, B., Piper, P.W. and Pearl, L.H. (2004) Structural basis for recruitment of the ATPase activator Aha1 to the Hsp90 chaperone machinery. *The EMBO journal*. **23**, 511–519 [CrossRef PubMed](#)
- 28 Voellmy, R. (2004) On mechanisms that control heat shock transcription factor activity in metazoan cells. *Cell stress & chaperones*. **9**, 122–133 [CrossRef](#)
- 29 Sorger, P.K. and Pelham, H.R. (1988) Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. *Cell*. **54**, 855–864 [CrossRef PubMed](#)
- 30 Pirkkala, L., Nykanen, P. and Sistonen, L. (2001) Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. **15**, 1118–1131 [CrossRef PubMed](#)
- 31 Wiederrecht, G., Seto, D. and Parker, C.S. (1988) Isolation of the gene encoding the *S. cerevisiae* heat shock transcription factor. *Cell*. **54**, 841–853 [CrossRef PubMed](#)
- 32 Sorger, P.K. (1991) Heat shock factor and the heat shock response. *Cell*. **65**, 363–366 [CrossRef PubMed](#)
- 33 Vergheze, J., Abrams, J., Wang, Y. and Morano, K.A. (2012) Biology of the heat shock response and protein chaperones: budding yeast (*Saccharomyces cerevisiae*) as a model system. *Microbiology and molecular biology reviews : MMBR*. **76**, 115–158 [CrossRef PubMed](#)
- 34 Anckar, J. and Sistonen, L. (2011) Regulation of HSF1 function in the heat stress response: implications in aging and disease. *Annual review of biochemistry*. **80**, 1089–1115 [CrossRef PubMed](#)
- 35 Holmberg, C.I., Tran, S.E., Eriksson, J.E. and Sistonen, L. (2002) Multisite phosphorylation provides sophisticated regulation of transcription factors. *Trends in biochemical sciences*. **27**, 619–627 [CrossRef PubMed](#)
- 36 Nieto-Sotelo, J., Wiederrecht, G., Okuda, A. and Parker, C.S. (1990) The yeast heat shock transcription factor contains a transcriptional activation domain whose activity is repressed under nonshock conditions. *Cell*. **62**, 807–817 [CrossRef PubMed](#)
- 37 Sakurai, H. and Enoki, Y. (2010) Novel aspects of heat shock factors: DNA recognition, chromatin modulation and gene expression. *The FEBS journal*. **277**, 4140–4149 [CrossRef PubMed](#)
- 38 Peteranderl, R., Rabenstein, M., Shin, Y.K., Liu, C.W., Wemmer, D.E., King, D.S. and Nelson, H.C. (1999) Biochemical and biophysical characterization of the trimerization domain from the heat shock transcription factor. *Biochemistry*. **38**, 3559–3569 [CrossRef PubMed](#)
- 39 Farkas, T., Kutsikova, Y.A. and Zimarino, V. (1998) Intramolecular repression of mouse heat shock factor 1. *Molecular and cellular biology*. **18**, 906–918 [CrossRef PubMed](#)
- 40 Rabinrand, S.K., Haroun, R.I., Clos, J., Wisniewski, J. and Wu, C. (1993) Regulation of heat shock factor trimer formation: role of a conserved leucine zipper. *Science*. **259**, 230–234 [CrossRef PubMed](#)
- 41 Zuo, J., Baler, R., Dahl, G. and Voellmy, R. (1994) Activation of the DNA-binding ability of human heat shock transcription factor 1 may involve the transition from an intramolecular to an intermolecular triple-stranded coiled-coil structure. *Molecular and cellular biology*. **14**, 7557–7568 [CrossRef PubMed](#)
- 42 Lu, M., Lee, Y.J., Park, S.M., Kang, H.S., Kang, S.W., Kim, S. and Park, J.S. (2009) Aromatic-participant interactions are essential for disulfide-bond-based trimerization in human heat shock transcription factor 1. *Biochemistry*. **48**, 3795–3797 [CrossRef PubMed](#)
- 43 Ahn, S.G. and Thiele, D.J. (2003) Redox regulation of mammalian heat shock factor 1 is essential for Hsp gene activation and protection from stress. *Genes & development*. **17**, 516–528 [CrossRef](#)
- 44 Bulman, A.L. and Nelson, H.C. (2005) Role of trehalose and heat in the structure of the C-terminal activation domain of the heat shock transcription factor. *Proteins*. **58**, 826–835 [CrossRef PubMed](#)
- 45 Pattaramanon, N., Sangha, N. and Gafni, A. (2007) The carboxy-terminal domain of heat-shock factor 1 is largely unfolded but can be induced to collapse into a compact, partially structured state. *Biochemistry*. **46**, 3405–3415 [CrossRef PubMed](#)
- 46 Hashikawa, N., Mizukami, Y., Imazu, H. and Sakurai, H. (2006) Mutated yeast heat shock transcription factor activates transcription independently of hyperphosphorylation. *The Journal of biological chemistry*. **281**, 3936–3942 [CrossRef PubMed](#)
- 47 Yamamoto, A., Ueda, J., Yamamoto, N., Hashikawa, N. and Sakurai, H. (2007) Role of heat shock transcription factor in *Saccharomyces cerevisiae* oxidative stress response. *Eukaryotic cell*. **6**, 1373–1379 [CrossRef PubMed](#)
- 48 Hashikawa, N., Yamamoto, N. and Sakurai, H. (2007) Different mechanisms are involved in the transcriptional activation by yeast heat shock transcription factor through two different types of heat shock elements. *The Journal of biological chemistry*. **282**, 10333–10340 [CrossRef PubMed](#)
- 49 Cho, H.S., Liu, C.W., Damberger, F.F., Pelton, J.G., Nelson, H.C. and Wemmer, D.E. (1996) Yeast heat shock transcription factor N-terminal activation domains are unstructured as probed by heteronuclear NMR spectroscopy. *Protein science : a publication of the Protein Society*. **5**, 262–269 [CrossRef PubMed](#)
- 50 Bonner, J.J., Heyward, S. and Fackenthal, D.L. (1992) Temperature-dependent regulation of a heterologous transcriptional activation domain fused to yeast heat shock transcription factor. *Molecular and cellular biology*. **12**, 1021–1030 [CrossRef PubMed](#)
- 51 Chen, T., Li, F. and Chen, B.S. (2009) Cross-talks of sensory transcription networks in response to various environmental stresses. *Interdisciplinary sciences, computational life sciences*. **1**, 46–54 [CrossRef PubMed](#)
- 52 Sorger, P.K. (1990) Yeast heat shock factor contains separable transient and sustained response transcriptional activators. *Cell*. **62**, 793–805 [CrossRef PubMed](#)
- 53 Ali, A., Bharadwaj, S., O'Carroll, R. and Ovsenek, N. (1998) HSP90 interacts with and regulates the activity of heat shock factor 1 in *Xenopus* oocytes. *Molecular and cellular biology*. **18**, 4949–4960 [CrossRef PubMed](#)
- 54 Bharadwaj, S., Ali, A. and Ovsenek, N. (1999) Multiple components of the HSP90 chaperone complex function in regulation of heat shock factor 1 In vivo. *Molecular and cellular biology*. **19**, 8033–8041 [CrossRef PubMed](#)
- 55 Zou, J., Guo, Y., Guettouche, T., Smith, D.F. and Voellmy, R. (1998) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell*. **94**, 471–480 [CrossRef PubMed](#)
- 56 Amin, J., Ananthan, J. and Voellmy, R. (1988) Key features of heat shock regulatory elements. *Molecular and cellular biology*. **8**, 3761–3769 [CrossRef PubMed](#)
- 57 Pelham, H.R. (1982) A regulatory upstream promoter element in the *Drosophila* hsp 70 heat-shock gene. *Cell*. **30**, 517–528 [CrossRef PubMed](#)
- 58 Xiao, H. and Lis, J.T. (1988) Germline transformation used to define key features of heat-shock response elements. *Science*. **239**, 1139–1142 [CrossRef PubMed](#)
- 59 Cotto, J.J., Kline, M. and Morimoto, R.I. (1996) Activation of heat shock factor 1 DNA binding precedes stress-induced serine phosphorylation. Evidence for a multistep pathway of regulation. *The Journal of biological chemistry*. **271**, 3355–3358
- 60 Jurivich, D.A., Sistonen, L., Kroes, R.A. and Morimoto, R.I. (1992) Effect of sodium salicylate on the human heat shock response. *Science*. **255**, 1243–1245 [CrossRef PubMed](#)
- 61 Zuo, J., Rungger, D. and Voellmy, R. (1995) Multiple layers of regulation of human heat shock transcription factor 1. *Molecular and cellular biology*. **15**, 4319–4330 [CrossRef PubMed](#)
- 62 Bruce, J.L., Price, B.D., Coleman, C.N. and Calderwood, S.K. (1993) Oxidative injury rapidly activates the heat shock transcription factor but fails to increase levels of heat shock proteins. *Cancer research*. **53**, 12–15 [PubMed](#)
- 63 Hensold, J.O., Hunt, C.R., Calderwood, S.K., Housman, D.E. and Kingston, R.E. (1990) DNA binding of heat shock factor to the heat shock element is insufficient for transcriptional activation in murine erythroleukemia cells. *Molecular and cellular biology*. **10**, 1600–1608 [CrossRef PubMed](#)
- 64 McConnell, J.R., Buckton, L.K. and McAlpine, S.R. (2015) Regulating the master regulator: Controlling heat shock factor 1 as a chemotherapy approach. *Bioorganic & medicinal chemistry letters*. **25**, 3409–3414 [CrossRef](#)
- 65 Heimberger, T., Andrusis, M., Riedel, S., Stuhmer, T., Schraud, H., Beilhack, A., Bumm, T., Bogen, B., Einsele, H., Bargou, R.C. and Chatterjee, M. (2013) The heat shock transcription factor 1 as a potential new therapeutic target in multiple myeloma. *British journal of haematology*. **160**, 465–476 [CrossRef PubMed](#)
- 66 Kline, M.P. and Morimoto, R.I. (1997) Repression of the heat shock factor 1 transcriptional activation domain is modulated by constitutive phosphorylation. *Molecular and cellular biology*. **17**, 2107–2115 [CrossRef PubMed](#)
- 67 Knauf, U., Newton, E.M., Kyriakis, J. and Kingston, R.E. (1996) Repression of human heat shock factor 1 activity at control temperature by phosphorylation. *Genes & development*. **10**, 2782–2793 [CrossRef](#)
- 68 Holmberg, C.I., Hietakangas, V., Mikhailov, A., Rantanen, J.O., Kallio, M., Meinander, A., Hellman, J., Morrice, N., MacKintosh, C., Morimoto, R.I., Eriksson, J.E. and Sistonen, L. (2001) Phosphorylation of serine 230 promotes inducible transcriptional activity of heat shock factor 1. *The EMBO journal*. **20**, 3800–3810 [CrossRef PubMed](#)
- 69 Boellmann, F., Guettouche, T., Guo, Y., Fenna, M., Mnayer, L. and Voellmy, R. (2004) DAXX interacts with heat shock factor 1 during stress activation and enhances its transcriptional activity. *Proceedings of the National Academy of Sciences of the United States of America*. **101**, 4100–4105 [CrossRef PubMed](#)
- 70 Budzynski, M.A., Puustinen, M.C., Joutsen, J. and Sistonen, L. (2015) Uncoupling Stress-Inducible Phosphorylation of Heat Shock Factor 1 from Its Activation. *Molecular and cellular biology*. **35**, 2530–2540 [CrossRef PubMed](#)
- 71 Anckar, J. and Sistonen, L. (2007) SUMO: getting it on. *Biochemical Society transactions*. **35**, 1409–1413 [CrossRef PubMed](#)
- 72 Hietakangas, V., Anckar, J., Blomster, H.A., Fujimoto, M., Palvimäki, J.J., Nakai, A. and Sistonen, L. (2006) PDSM, a motif for phosphorylation-dependent SUMO modification. *Proceedings of the National Academy of Sciences of the United States of America*. **103**, 45–50 [CrossRef PubMed](#)
- 73 Geiss-Friedlander, R. and Melchior, F. (2007) Concepts in sumoylation: a decade on. *Nature reviews. Molecular cell biology*. **8**, 947–956 [CrossRef PubMed](#)

- 74 Newton, E.M., Knauf, U., Green, M. and Kingston, R.E. (1996) The regulatory domain of human heat shock factor 1 is sufficient to sense heat stress. *Molecular and cellular biology*. **16**, 839–846 [CrossRef PubMed](#)
- 75 Westerheide, S.D., Ancker, J., Stevens, S.M., Jr., Sistonen, L. and Morimoto, R.I. (2009) Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. *Science*. **323**, 1063–1066 [CrossRef PubMed](#)
- 76 Liu, P.C. and Thiele, D.J. (1999) Modulation of human heat shock factor trimerization by the linker domain. *The Journal of biological chemistry*. **274**, 17219–17225 [CrossRef PubMed](#)
- 77 Vujanac, M., Fenaroli, A. and Zimarino, V. (2005) Constitutive nuclear import and stress-regulated nucleocytoplasmic shuttling of mammalian heat-shock factor 1. *Traffic*. **6**, 214–229 [CrossRef PubMed](#)
- 78 Brown, S.A., Weirich, C.S., Newton, E.M. and Kingston, R.E. (1998) Transcriptional activation domains stimulate initiation and elongation at different times and via different residues. *The EMBO journal*. **17**, 3146–3154 [CrossRef PubMed](#)
- 79 Morimoto, R.I. (2002) Dynamic remodeling of transcription complexes by molecular chaperones. *Cell*. **110**, 281–284 [CrossRef PubMed](#)
- 80 Shi, Y., Mosser, D.D. and Morimoto, R.I. (1998) Molecular chaperones as HSF1-specific transcriptional repressors. *Genes & development*. **12**, 654–666 [CrossRef](#)
- 81 Gomez, A.V., Galleguillos, D., Maass, J.C., Battaglioli, E., Kukuljan, M. and Andres, M.E. (2008) CoREST represses the heat shock response mediated by HSF1. *Molecular cell*. **31**, 222–231 [CrossRef PubMed](#)
- 82 Shamovsky, I., Ivannikov, M., Kandel, E.S., Gershon, D. and Nudler, E. (2006) RNA-mediated response to heat shock in mammalian cells. *Nature*. **440**, 556–560 [CrossRef PubMed](#)
- 83 Kugel, J.F. and Goodrich, J.A. (2006) Beating the heat: A translation factor and an RNA mobilize the heat shock transcription factor HSF1. *Molecular cell*. **22**, 153–154 [CrossRef PubMed](#)
- 84 Neef, D.W., Turski, M.L. and Thiele, D.J. (2010) Modulation of heat shock transcription factor 1 as a therapeutic target for small molecule intervention in neurodegenerative disease. *PLoS biology*. **8**, e1000291 [CrossRef PubMed](#)
- 85 Chen, B., Piel, W.H., Gui, L., Bruford, E. and Monteiro, A. (2005) The HSP90 family of genes in the human genome: insights into their divergence and evolution. *Genomics*. **86**, 627–637 [CrossRef PubMed](#)
- 86 Hickey, E., Brandon, S.E., Smale, G., Lloyd, D. and Weber, L.A. (1989) Sequence and regulation of a gene encoding a human 89-kilodalton heat shock protein. *Mol Cell Biol*. **9**, 2615–2626 [CrossRef PubMed](#)
- 87 Rebbe, N.F., Hickman, W.S., Ley, T.J., Stafford, D.W. and Hickman, S. (1989) Nucleotide sequence and regulation of a human 90-kDa heat shock protein gene. *J Biol Chem*. **264**, 15006–15011 [PubMed](#)
- 88 Shen, Y., Liu, J., Wang, X., Cheng, X., Wang, Y. and Wu, N. (1997) Essential role of the first intron in the transcription of hsp90beta gene. *FEBS letters*. **413**, 92–98 [CrossRef PubMed](#)
- 89 Zhang, S.L., Yu, J., Cheng, X.K., Ding, L., Heng, F.Y., Wu, N.H. and Shen, Y.F. (1999) Regulation of human hsp90alpha gene expression. *FEBS letters*. **444**, 130–135 [CrossRef PubMed](#)
- 90 Stephanou, A. and Latchman, D.S. (1999) Transcriptional regulation of the heat shock protein genes by STAT family transcription factors. *Gene expression*. **7**, 311–319 [PubMed](#)
- 91 Stephanou, A., Isenberg, D.A., Nakajima, K. and Latchman, D.S. (1999) Signal transducer and activator of transcription-1 and heat shock factor-1 interact and activate the transcription of the Hsp-70 and Hsp-90beta gene promoters. *J Biol Chem*. **274**, 1723–1728 [CrossRef PubMed](#)
- 92 Ehret, G.B., Reichenbach, P., Schindler, U., Horvath, C.M., Fritz, S., Nabholz, M. and Bucher, P. (2001) DNA binding specificity of different STAT proteins. Comparison of in vitro specificity with natural target sites. *J Biol Chem*. **276**, 6675–6688 [CrossRef PubMed](#)
- 93 Combates, N.J., Rzepka, R.W., Chen, Y.N. and Cohen, D. (1994) NF-IL6, a member of the C/EBP family of transcription factors, binds and trans-activates the human MDR1 gene promoter. *J Biol Chem*. **269**, 29715–29719 [PubMed](#)
- 94 Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T. and Kishimoto, T. (1990) A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. *EMBO J*. **9**, 1897–1906 [PubMed](#)
- 95 Xu, D., Zalmas, L.P. and La Thangue, N.B. (2008) A transcription cofactor required for the heat-shock response. *EMBO reports*. **9**, 662–669 [CrossRef PubMed](#)
- 96 Barlev, N.A., Liu, L., Chehab, N.H., Mansfield, K., Harris, K.G., Halazonetis, T.D. and Berger, S.L. (2001) Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. *Molecular cell*. **8**, 1243–1254 [CrossRef PubMed](#)
- 97 Espinosa, J.M. and Emerson, B.M. (2001) Transcriptional regulation by p53 through intrinsic DNA/chromatin binding and site-directed cofactor recruitment. *Molecular cell*. **8**, 57–69 [CrossRef PubMed](#)
- 98 Ito, A., Lai, C.H., Zhao, X., Saito, S., Hamilton, M.H., Appella, E. and Yao, T.P. (2001) p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2. *The EMBO journal*. **20**, 1331–1340 [CrossRef PubMed](#)
- 99 Ghosh, S., May, M.J. and Kopp, E.B. (1998) NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annual review of immunology*. **16**, 225–260 [CrossRef PubMed](#)
- 100 Li, Q. and Verma, I.M. (2002) NF-kappaB regulation in the immune system. *Nature reviews. Immunology*. **2**, 725–734 [CrossRef PubMed](#)
- 101 Bonizzi, G. and Karin, M. (2004) The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends in immunology*. **25**, 280–288 [CrossRef PubMed](#)
- 102 Gerondakis, S., Fulford, T.S., Messina, N.L. and Grumont, R.J. (2014) NF-kappaB control of T cell development. *Nature immunology*. **15**, 15–25 [CrossRef PubMed](#)
- 103 Gugasyan, R., Voss, A., Varigos, G., Thomas, T., Grumont, R.J., Kaur, P., Grigoriadis, G. and Gerondakis, S. (2004) The transcription factors c-rel and RelA control epidermal development and homeostasis in embryonic and adult skin via distinct mechanisms. *Mol Cell Biol*. **24**, 5733–5745 [CrossRef PubMed](#)
- 104 Oeckinghaus, A., Hayden, M.S. and Ghosh, S. (2011) Crosstalk in NF-kappaB signaling pathways. *Nature immunology*. **12**, 695–708 [CrossRef PubMed](#)
- 105 Smale, S.T. (2011) Hierarchies of NF-kappaB target-gene regulation. *Nature immunology*. **12**, 689–694 [CrossRef PubMed](#)
- 106 Napetschnig, J. and Wu, H. (2013) Molecular basis of NF-kappaB signaling. *Annual review of biophysics*. **42**, 443–468 [CrossRef PubMed](#)
- 107 Broemer, M., Krappmann, D. and Scheidereit, C. (2004) Requirement of Hsp90 activity for IkkappaB kinase (IKK) biosynthesis and for constitutive and inducible IKK and NF-kappaB activation. *Oncogene*. **23**, 5378–5386 [CrossRef PubMed](#)
- 108 Chen, G., Cao, P. and Goeddel, D.V. (2002) TNF-induced recruitment and activation of the IKK complex require Cdc37 and Hsp90. *Molecular cell*. **9**, 401–410 [CrossRef PubMed](#)
- 109 Field, N., Low, W., Daniels, M., Howell, S., Daviet, L., Boshoff, C. and Collins, M. (2003) KSHV vFLIP binds to IKK-gamma to activate IKK. *Journal of cell science*. **116**, 3721–3728 [CrossRef PubMed](#)
- 110 Mitsiades, C.S., Mitsiades, N.S., McMullan, C.J., Poulaki, V., Kung, A.L., Davies, F.E., Morgan, G., Akiyama, M., Shringarpure, R., Munshi, N.C., Richardson, P.G., Hideshima, T., Chauhan, D., Gu, X., Bailey, C., Joseph, M., Libermann, T.A., Rosen, N.S. and Anderson, K.C. (2006) Antimyeloma activity of heat shock protein-90 inhibition. *Blood*. **107**, 1092–1100 [CrossRef PubMed](#)
- 111 Pittet, J.F., Lee, H., Pespeni, M., O'Mahony, A., Roux, J. and Welch, W.J. (2005) Stress-induced inhibition of the NF-kappaB signaling pathway results from the insolubilization of the IkkappaB kinase complex following its dissociation from heat shock protein 90. *Journal of immunology*. **174**, 384–394 [CrossRef](#)
- 112 Mollapour, M. and Neckers, L. (2012) Post-translational modifications of Hsp90 and their contributions to chaperone regulation. *Biochimica et biophysica acta*. **1823**, 648–655 [CrossRef PubMed](#)
- 113 Quanz, M., Herbette, A., Sayarath, M., de Koning, L., Dubois, T., Sun, J.S. and Dutreix, M. (2012) Heat shock protein 90alpha (Hsp90alpha) is phosphorylated in response to DNA damage and accumulates in repair foci. *The Journal of biological chemistry*. **287**, 8803–8815 [CrossRef PubMed](#)
- 114 Solier, S., Kohn, K.W., Scroggins, B., Xu, W., Trepel, J., Neckers, L. and Pommier, Y. (2012) Heat shock protein 90alpha (HSP90alpha), a substrate and chaperone of DNA-PK necessary for the apoptotic response. *Proceedings of the National Academy of Sciences of the United States of America*. **109**, 12866–12872 [CrossRef PubMed](#)
- 115 Mollapour, M., Tsutsumi, S. and Neckers, L. (2010) Hsp90 phosphorylation, Wee1 and the cell cycle. *Cell cycle*. **9**, 2310–2316 [CrossRef PubMed](#)
- 116 Legagneux, V., Morange, M. and Bensaude, O. (1991) Heat shock increases turnover of 90 kDa heat shock protein phosphate groups in HeLa cells. *FEBS letters*. **291**, 359–362 [CrossRef PubMed](#)
- 117 Zhao, Y.G., Gilmore, R., Leone, G., Coffey, M.C., Weber, B. and Lee, P.W. (2001) Hsp90 phosphorylation is linked to its chaperoning function. Assembly of the reovirus cell attachment protein. *The Journal of biological chemistry*. **276**, 32822–32827 [CrossRef PubMed](#)
- 118 Xu, W., Mollapour, M., Prodromou, C., Wang, S., Scroggins, B.T., Palchick, Z., Beebe, K., Siderius, M., Lee, M.J., Couvillon, A., Trepel, J.B., Miyata, Y., Matts, R. and Neckers, L. (2012) Dynamic tyrosine phosphorylation modulates cycling of the HSP90-P50(CDC37)-AHA1 chaperone machine. *Molecular cell*. **47**, 434–443 [CrossRef PubMed](#)
- 119 Muller, P., Ruckova, E., Halada, P., Coates, P.J., Hrstka, R., Lane, D.P. and Vojtesek, B. (2013) C-terminal phosphorylation of Hsp70 and Hsp90 regulates alternate binding to co-chaperones CHIP and HOP to determine cellular protein folding/degradation balances. *Oncogene*. **32**, 3101–3110 [CrossRef PubMed](#)
- 120 Mollapour, M., Tsutsumi, S., Truman, A.W., Xu, W., Vaughan, C.K., Beebe, K., Konstantinova, A., Vourganti, S., Panaretou, B., Piper, P.W., Trepel, J.B., Prodromou, C., Pearl, L.H. and Neckers, L. (2011) Threonine 22 phosphorylation attenuates Hsp90 interaction with cochaperones and affects its chaperone activity. *Molecular cell*. **41**, 672–681 [CrossRef PubMed](#)

- 121 Mollapour, M., Tsutsumi, S., Kim, Y.S., Trepel, J. and Neckers, L. (2011) Casein kinase 2 phosphorylation of Hsp90 threonine 22 modulates chaperone function and drug sensitivity. *Oncotarget*. **2**, 407–417 [CrossRef PubMed](#)
- 122 Deb, T.B., Zuo, A.H., Wang, Y., Berndt, R.J., Cheema, A.K., Sengupta, S., Coticchia, C.M. and Johnson, M.D. (2011) Pnck induces ligand-independent EGFR degradation by probable perturbation of the Hsp90 chaperone complex. *American journal of physiology. Cell physiology*. **300**, C1139–1154 [CrossRef PubMed](#)
- 123 Kettenbach, A.N., Schweppe, D.K., Faherty, B.K., Pechenick, D., Pletnev, A.A. and Gerber, S.A. (2011) Quantitative phosphoproteomics identifies substrates and functional modules of Aurora and Polo-like kinase activities in mitotic cells. *Science signaling*. **4**, rs5 [CrossRef PubMed](#)
- 124 Lei, H., Venkatakrishnan, A., Yu, S. and Kazlauskas, A. (2007) Protein kinase A-dependent translocation of Hsp90 alpha impairs endothelial nitric-oxide synthase activity in high glucose and diabetes. *The Journal of biological chemistry*. **282**, 9364–9371 [CrossRef PubMed](#)
- 125 Wang, X., Song, X., Zhuo, W., Fu, Y., Shi, H., Liang, Y., Tong, M., Chang, G. and Luo, Y. (2009) The regulatory mechanism of Hsp90alpha secretion and its function in tumor malignancy. *Proceedings of the National Academy of Sciences of the United States of America*. **106**, 21288–21293 [CrossRef PubMed](#)
- 126 Ogiso, H., Kagi, N., Matsumoto, E., Nishimoto, M., Arai, R., Shirouzu, M., Mimura, J., Fujii-Kuriyama, Y. and Yokoyama, S. (2004) Phosphorylation analysis of 90 kDa heat shock protein within the cytosolic arylhydrocarbon receptor complex. *Biochemistry*. **43**, 15510–15519 [CrossRef PubMed](#)
- 127 Wandering, S.K., Suhre, M.H., Wegele, H. and Buchner, J. (2006) The phosphatase Ppt1 is a dedicated regulator of the molecular chaperone Hsp90. *The EMBO journal*. **25**, 367–376 [CrossRef PubMed](#)
- 128 Bali, P., Pranpat, M., Bradner, J., Balasis, M., Fiskus, W., Guo, F., Rocha, K., Kumaraswamy, S., Boyapalle, S., Atadja, P., Seto, E. and Bhalla, K. (2005) Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors. *The Journal of biological chemistry*. **280**, 26729–26734 [CrossRef PubMed](#)
- 129 Kekatpure, V.D., Dannenberg, A.J. and Subbaramaiah, K. (2009) HDAC6 modulates Hsp90 chaperone activity and regulates activation of aryl hydrocarbon receptor signaling. *The Journal of biological chemistry*. **284**, 7436–7445 [CrossRef PubMed](#)
- 130 Murphy, P.J., Morishima, Y., Kovacs, J.J., Yao, T.P. and Pratt, W.B. (2005) Regulation of the dynamics of hsp90 action on the glucocorticoid receptor by acetylation/deacetylation of the chaperone. *The Journal of biological chemistry*. **280**, 33792–33799 [CrossRef PubMed](#)
- 131 Park, J.H., Kim, S.H., Choi, M.C., Lee, J., Oh, D.Y., Im, S.A., Bang, Y.J. and Kim, T.Y. (2008) Class II histone deacetylases play pivotal roles in heat shock protein 90-mediated proteasomal degradation of vascular endothelial growth factor receptors. *Biochemical and biophysical research communications*. **368**, 318–322 [CrossRef PubMed](#)
- 132 Zhou, Q., Agoston, A.T., Atadja, P., Nelson, W.G. and Davidson, N.E. (2008) Inhibition of histone deacetylases promotes ubiquitin-dependent proteasomal degradation of DNA methyltransferase 1 in human breast cancer cells. *Molecular cancer research : MCR*. **6**, 873–883 [CrossRef PubMed](#)
- 133 Yang, Y., Rao, R., Shen, J., Tang, Y., Fiskus, W., Nechtman, J., Atadja, P. and Bhalla, K. (2008) Role of acetylation and extracellular location of heat shock protein 90alpha in tumor cell invasion. *Cancer research*. **68**, 4833–4842 [CrossRef PubMed](#)
- 134 Scroggins, B.T., Robzyk, K., Wang, D., Marcu, M.G., Tsutsumi, S., Beebe, K., Cotter, R.J., Felts, S., Toft, D., Karnitz, L., Rosen, N. and Neckers, L. (2007) An acetylation site in the middle domain of Hsp90 regulates chaperone function. *Molecular cell*. **25**, 151–159 [CrossRef PubMed](#)
- 135 Martinez-Ruiz, A., Villanueva, L., Gonzalez de Orduna, C., Lopez-Ferrer, D., Higuera, M.A., Tarin, C., Rodriguez-Crespo, I., Vazquez, J. and Lamas, S. (2005) S-nitrosylation of Hsp90 promotes the inhibition of its ATPase and endothelial nitric oxide synthase regulatory activities. *Proceedings of the National Academy of Sciences of the United States of America*. **102**, 8525–8530 [CrossRef PubMed](#)
- 136 Retzlaff, M., Stahl, M., Eberl, H.C., Lagleder, S., Beck, J., Kessler, H. and Buchner, J. (2009) Hsp90 is regulated by a switch point in the C-terminal domain. *EMBO reports*. **10**, 1147–1153 [CrossRef PubMed](#)
- 137 Blank, M., Mandel, M., Keisari, Y., Meruelo, D. and Lavie, G. (2003) Enhanced ubiquitinylation of heat shock protein 90 as a potential mechanism for mitotic cell death in cancer cells induced with hypericin. *Cancer research*. **63**, 8241–8247 [PubMed](#)
- 138 Murtagh, J., Lu, H. and Schwartz, E.L. (2006) Taxotere-induced inhibition of human endothelial cell migration is a result of heat shock protein 90 degradation. *Cancer research*. **66**, 8192–8199 [CrossRef PubMed](#)
- 139 Mollapour, M., Tsutsumi, S., Donnelly, A.C., Beebe, K., Tokita, M.J., Lee, M.J., Lee, S., Morra, G., Bourbouli, D., Scroggins, B.T., Colombo, G., Blagg, B.S., Panaretou, B., Stetler-Stevenson, W.G., Trepel, J.B., Piper, P.W., Prodromou, C., Pearl, L.H. and Neckers, L. (2010) Swe1Wee1-dependent tyrosine phosphorylation of Hsp90 regulates distinct facets of chaperone function. *Molecular cell*. **37**, 333–343 [CrossRef PubMed](#)
- 140 Chen, W.Y., Chang, F.R., Huang, Z.Y., Chen, J.H., Wu, Y.C. and Wu, C.C. (2008) Tubocapsenolide A, a novel withanolide, inhibits proliferation and induces apoptosis in MDA-MB-231 cells by thiol oxidation of heat shock proteins. *The Journal of biological chemistry*. **283**, 17184–17193 [CrossRef PubMed](#)
- 141 Mollapour, M., Bourbouli, D., Beebe, K., Woodford, M.R., Polier, S., Hoang, A., Chelluri, R., Li, Y., Guo, A., Lee, M.J., Fotooh-Abadi, E., Khan, S., Prince, T., Miyajima, N., Yoshida, S., Tsutsumi, S., Xu, W., Panaretou, B., Stetler-Stevenson, W.G., Bratslavsky, G., Trepel, J.B., Prodromou, C. and Neckers, L. (2014) Asymmetric Hsp90 N domain SUMOylation recruits Aha1 and ATP-competitive inhibitors. *Molecular cell*. **53**, 317–329 [CrossRef PubMed](#)
- 142 Jakob, U., Scheibel, T., Bose, S., Reinstein, J. and Buchner, J. (1996) Assessment of the ATP binding properties of Hsp90. *The Journal of biological chemistry*. **271**, 10035–10041 [CrossRef PubMed](#)
- 143 Smith, D.F., Stensgard, B.A., Welch, W.J. and Toft, D.O. (1992) Assembly of progesterone receptor with heat shock proteins and receptor activation are ATP mediated events. *The Journal of biological chemistry*. **267**, 1350–1356 [PubMed](#)
- 144 Csermely, P., Kajtar, J., Hollósi, M., Jalsovszky, G., Holly, S., Kahn, C.R., Gergely, P., Jr., Soti, C., Mihaly, K. and Somogyi, J. (1993) ATP induces a conformational change of the 90-kDa heat shock protein (hsp90). *The Journal of biological chemistry*. **268**, 1901–1907 [PubMed](#)
- 145 Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W. and Pearl, L.H. (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell*. **90**, 65–75 [CrossRef PubMed](#)
- 146 Vaughan, C.K., Piper, P.W., Pearl, L.H. and Prodromou, C. (2009) A common conformationally coupled ATPase mechanism for yeast and human cytoplasmic HSP90s. *The FEBS journal*. **276**, 199–209 [CrossRef PubMed](#)
- 147 Frey, S., Leskovaar, A., Reinstein, J. and Buchner, J. (2007) The ATPase cycle of the endoplasmic chaperone Grp94. *The Journal of biological chemistry*. **282**, 35612–35620 [CrossRef PubMed](#)
- 148 Leskovaar, A., Wegele, H., Werbeck, N.D., Buchner, J. and Reinstein, J. (2008) The ATPase cycle of the mitochondrial Hsp90 analog Trap1. *The Journal of biological chemistry*. **283**, 11677–11688 [CrossRef PubMed](#)
- 149 Panaretou, B., Siligardi, G., Meyer, P., Maloney, A., Sullivan, J.K., Singh, S., Millson, S.H., Clarke, P.A., Naaby-Hansen, S., Stein, R., Cramer, R., Mollapour, M., Workman, P., Piper, P.W., Pearl, L.H. and Prodromou, C. (2002) Activation of the ATPase activity of hsp90 by the stress-regulated cochaperone aha1. *Molecular cell*. **10**, 1307–1318 [CrossRef PubMed](#)
- 150 Siligardi, G., Hu, B., Panaretou, B., Piper, P.W., Pearl, L.H. and Prodromou, C. (2004) Co-chaperone regulation of conformational switching in the Hsp90 ATPase cycle. *The Journal of biological chemistry*. **279**, 51989–51998 [CrossRef PubMed](#)
- 151 Weikl, T., Muschler, P., Richter, K., Veit, T., Reinstein, J. and Buchner, J. (2000) C-terminal regions of Hsp90 are important for trapping the nucleotide during the ATPase cycle. *Journal of molecular biology*. **303**, 583–592 [CrossRef PubMed](#)
- 152 Graf, C., Stankiewicz, M., Kramer, G. and Mayer, M.P. (2009) Spatially and kinetically resolved changes in the conformational dynamics of the Hsp90 chaperone machine. *The EMBO journal*. **28**, 602–613 [CrossRef PubMed](#)
- 153 Hesslering, M., Richter, K. and Buchner, J. (2009) Dissection of the ATP-induced conformational cycle of the molecular chaperone Hsp90. *Nature Structural & Molecular Biology*. **16**, 287–293 [CrossRef](#)
- 154 Shiau, A.K., Harris, S.F., Southworth, D.R. and Agard, D.A. (2006) Structural Analysis of E. coli hsp90 Reveals Dramatic Nucleotide-Dependent Conformational Rearrangements. *Cell*. **127**, 329–340 [CrossRef PubMed](#)
- 155 Pearl, L.H. and Prodromou, C. (2006) Structure and mechanism of the Hsp90 molecular chaperone machinery. *Annual review of biochemistry*. **75**, 271–294 [CrossRef PubMed](#)
- 156 Schuler, B. and Hofmann, H. (2013) Single-molecule spectroscopy of protein folding dynamics-expanding scope and timescales. *Current opinion in structural biology*. **23**, 36–47 [CrossRef PubMed](#)
- 157 Cunningham, C.N., Southworth, D.R., Krukenberg, K.A. and Agard, D.A. (2012) The conserved arginine 380 of Hsp90 is not a catalytic residue, but stabilizes the closed conformation required for ATP hydrolysis. *Protein Science*. **21**, 1162–1171 [CrossRef](#)
- 158 Prodromou, C., Siligardi, G., O'Brien, R., Woolfson, D.N., Regan, L., Panaretou, B., Ladbury, J.E., Piper, P.W. and Pearl, L.H. (1999) Regulation of Hsp90 ATPase activity by tetratricopeptide repeat (TPR)-domain co-chaperones. *The EMBO journal*. **18**, 754–762 [CrossRef PubMed](#)
- 159 Li, J., Richter, K. and Buchner, J. (2011) Mixed Hsp90-cochaperone complexes are important for the progression of the reaction cycle. *Nature structural & molecular biology*. **18**, 61–66 [CrossRef](#)
- 160 Scheuffer, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., Hartl, F.U. and Moarefi, I. (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell*. **101**, 199–210 [CrossRef PubMed](#)

- 161 Onuoha, S.C., Coulstock, E.T., Grossmann, J.G. and Jackson, S.E. (2008) Structural studies on the co-chaperone Hop and its complexes with Hsp90. *J Mol Biol.* **379**, 732–744 [CrossRef PubMed](#)
- 162 Richter, K., Muschler, P., Hainzl, O., Reinstein, J. and Buchner, J. (2003) Sti1 is a non-competitive inhibitor of the Hsp90 ATPase. Binding prevents the N-terminal dimerization reaction during the atpase cycle. *J Biol Chem.* **278**, 10328–10333 [CrossRef PubMed](#)
- 163 Southworth, D.R. and Agard, D.A. (2011) Client-Loading Conformation of the Hsp90 Molecular Chaperone Revealed in the Cryo-EM Structure of the Human Hsp90:Hop Complex. *Mol Cell.* **42**, 771–781 [CrossRef PubMed](#)
- 164 Siligardi, G., Panaretou, B., Meyer, P., Singh, S., Woolfson, D.N., Piper, P.W., Pearl, L.H. and Prodromou, C. (2002) Regulation of Hsp90 ATPase activity by the co-chaperone Cdc37p/p50cdc37. *The Journal of biological chemistry.* **277**, 20151–20159 [CrossRef PubMed](#)
- 165 Roe, S.M., Ali, M.M., Meyer, P., Vaughan, C.K., Panaretou, B., Piper, P.W., Prodromou, C. and Pearl, L.H. (2004) The Mechanism of Hsp90 regulation by the protein kinase-specific cochaperone p50(cdc37). *Cell* **116**, 87–98 [CrossRef PubMed](#)
- 166 Takahashi, A., Casais, C., Ichimura, K. and Shirasu, K. (2003) HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in Arabidopsis. *Proc Natl Acad Sci U S A.* **100**, 11777–11782 [CrossRef PubMed](#)
- 167 Hubert, D.A., Tornero, P., Belkadir, Y., Krishna, P., Takahashi, A., Shirasu, K. and Dangl, J.L. (2003) Cytosolic HSP90 associates with and modulates the Arabidopsis RPM1 disease resistance protein. *EMBO J.* **22**, 5679–5689 [CrossRef PubMed](#)
- 168 Liu, Y., Burch-Smith, T., Schiff, M., Feng, S. and Dinesh-Kumar, S.P. (2004) Molecular chaperone Hsp90 associates with resistance protein N and its signaling proteins SGT1 and Rar1 to modulate an innate immune response in plants. *J Biol Chem.* **279**, 2101–2108 [CrossRef PubMed](#)
- 169 Zhang, M., Boter, M., Li, K., Kadota, Y., Panaretou, B., Prodromou, C., Shirasu, K. and Pearl, L.H. (2008) Structural and functional coupling of Hsp90- and Sgt1-centred multi-protein complexes. *The EMBO journal.* **27**, 2789–2798 [CrossRef PubMed](#)
- 170 Zhang, M., Kadota, Y., Prodromou, C., Shirasu, K. and Pearl, L.H. (2010) Structural basis for assembly of Hsp90-Sgt1-CHORD protein complexes: implications for chaperoning of NLR innate immunity receptors. *Molecular cell.* **39**, 269–281 [CrossRef PubMed](#)
- 171 Sbroglio, M., Ferretti, R., Percivalle, E., Gutkowska, M., Zyllicz, A., Michowski, W., Kuznicki, J., Accornero, F., Pacchioni, B., Lanfranchi, G., Hamm, J., Turco, E., Silengo, L., Tarone, G. and Brancaccio, M. (2008) The mammalian CHORD-containing protein melusin is a stress response protein interacting with Hsp90 and Sgt1. *FEBS Lett.* **582**, 1788–1794 [CrossRef PubMed](#)
- 172 Kadota, Y., Amigues, B., Ducassou, L., Madaoui, H., Ochsenbein, F., Guerois, R. and Shirasu, K. (2008) Structural and functional analysis of SGT1-HSP90 core complex required for innate immunity in plants. *EMBO Rep* **9**, 1209–1215 [CrossRef PubMed](#)
- 173 Shirasu, K. (2009) The HSP90-SGT1 chaperone complex for NLR immune sensors. *Annu Rev Plant Biol.* **60**, 139–164 [CrossRef PubMed](#)
- 174 Bansal, P.K., Nourse, A., Abdulle, R. and Kitagawa, K. (2009) Sgt1 dimerization is required for yeast kinetochore assembly. *J Biol Chem.* **284**, 3586–3592 [CrossRef PubMed](#)
- 175 Bansal, P.K., Abdulle, R. and Kitagawa, K. (2004) Sgt1 associates with Hsp90: an initial step of assembly of the core kinetochore complex. *Mol Cell Biol.* **24**, 8069–8079 [CrossRef PubMed](#)
- 176 Steensgaard, P., Garre, M., Muradore, I., Transidico, P., Nigg, E.A., Kitagawa, K., Earnshaw, W.C., Faretta, M. and Musacchio, A. (2004) Sgt1 is required for human kinetochore assembly. *EMBO Rep.* **5**, 626–631 [CrossRef PubMed](#)
- 177 Kitagawa, K., Skowrya, D., Elledge, S.J., Harper, J.W. and Hieter, P. (1999) SGT1 encodes an essential component of the yeast kinetochore assembly pathway and a novel subunit of the SCF ubiquitin ligase complex. *Mol Cell.* **4**, 21–33 [CrossRef PubMed](#)
- 178 Azevedo, C., Sadanandom, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K. and Schulze-Lefert, P. (2002) The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. *Science.* **295**, 2073–2076 [CrossRef PubMed](#)
- 179 Mayor, A., Martinon, F., De Smedt, T., Petrilli, V. and Tschopp, J. (2007) A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses. *Nat Immunol* **8**, 497–503 [CrossRef PubMed](#)
- 180 da Silva Correia, J., Miranda, Y., Leonard, N. and Ulevitch, R. (2007) SGT1 is essential for Nod1 activation. *Proc Natl Acad Sci U S A.* **104**, 6764–6769 [CrossRef PubMed](#)
- 181 Lingelbach, L.B. and Kaplan, K.B. (2004) The interaction between Sgt1p and Skp1p is regulated by HSP90 chaperones and is required for proper CBF3 assembly. *Mol Cell Biol.* **24**, 8938–8950 [CrossRef PubMed](#)
- 182 Catlett, M.G. and Kaplan, K.B. (2006) Sgt1p is a unique co-chaperone that acts as a client adaptor to link Hsp90 to Skp1p. *J Biol Chem.* **281**, 33739–33748 [CrossRef PubMed](#)
- 183 Meyer, P., Prodromou, C., Liao, C., Hu, B., Roe, S.M., Vaughan, C.K., Vlastic, I., Panaretou, B., Piper, P.W. and Pearl, L.H. (2004) Structural basis for recruitment of the ATPase activator Aha1 to the Hsp90 chaperone machinery. *EMBO J.* **23**, 1402–1410 [CrossRef PubMed](#)
- 184 Retzlaff, M., Hagn, F., Mitschke, L., Hessling, M., Gugel, F., Kessler, H., Richter, K. and Buchner, J. (2010) Asymmetric activation of the hsp90 dimer by its cochaperone aha1. *Molecular cell.* **37**, 344–354 [CrossRef PubMed](#)
- 185 McLaughlin, S.H., Sobott, F., Yao, Z.P., Zhang, W., Nielsen, P.R., Grossmann, J.G., Laue, E.D., Robinson, C.V. and Jackson, S.E. (2006) The co-chaperone p23 arrests the Hsp90 ATPase cycle to trap client proteins. *J Mol Biol.* **356**, 746–758 [CrossRef PubMed](#)
- 186 Miyata, Y. (2009) Protein kinase CK2 in health and disease: CK2: the kinase controlling the Hsp90 chaperone machinery. *Cellular and molecular life sciences : CMLS.* **66**, 1840–1849 [CrossRef PubMed](#)
- 187 Shao, J., Prince, T., Hartson, S.D. and Matts, R.L. (2003) Phosphorylation of serine 13 is required for the proper function of the Hsp90 co-chaperone, Cdc37. *The Journal of biological chemistry.* **278**, 38117–38120 [CrossRef PubMed](#)
- 188 Bandhakavi, S., McCann, R.O., Hanna, D.E. and Glover, C.V. (2003) A positive feedback loop between protein kinase CKII and Cdc37 promotes the activity of multiple protein kinases. *The Journal of biological chemistry.* **278**, 2829–2836 [CrossRef PubMed](#)
- 189 Vaughan, C.K., Mollapour, M., Smith, J.R., Truman, A., Hu, B., Good, V.M., Panaretou, B., Neckers, L., Clarke, P.A., Workman, P., Piper, P.W., Prodromou, C. and Pearl, L.H. (2008) Hsp90-dependent activation of protein kinases is regulated by chaperone-targeted dephosphorylation of Cdc37. *Molecular cell.* **31**, 886–895 [CrossRef PubMed](#)
- 190 Bansal, P.K., Mishra, A., High, A.A., Abdulle, R. and Kitagawa, K. (2009) Sgt1 dimerization is negatively regulated by protein kinase CK2-mediated phosphorylation at Ser361. *The Journal of biological chemistry.* **284**, 18692–18698 [CrossRef PubMed](#)
- 191 Kobayashi, T., Nakatani, Y., Tanioka, T., Tsujimoto, M., Nakajo, S., Nakaya, K., Murakami, M. and Kudo, I. (2004) Regulation of cytosolic prostaglandin E synthase by phosphorylation. *The Biochemical journal.* **381**, 59–69 [CrossRef PubMed](#)
- 192 Johnson, J.L. and Toft, D.O. (1994) A novel chaperone complex for steroid receptors involving heat shock proteins, immunophilins, and p23. *The Journal of biological chemistry.* **269**, 24989–24993 [PubMed](#)
- 193 Holt, S.E., Aisner, D.L., Baur, J., Tesmer, V.M., Dy, M., Ouellette, M., Trager, J.B., Morin, G.B., Toft, D.O., Shay, J.W., Wright, W.E. and White, M.A. (1999) Functional requirement of p23 and Hsp90 in telomerase complexes. *Genes & development.* **13**, 817–826 [CrossRef](#)
- 194 Longshaw, V.M., Dirr, H.W., Blatch, G.L. and Lassle, M. (2000) The in vitro phosphorylation of the co-chaperone mST11 by cell cycle kinases substantiates a predicted casein kinase II-p34cdc2-NLS (CcN) motif. *Biological chemistry.* **381**, 1133–1138 [CrossRef PubMed](#)
- 195 Miyata, Y., Chambrud, B., Radanyi, C., Leclerc, J., Lebeau, M.C., Renoir, J.M., Shirai, R., Catelli, M.G., Yahara, I. and Baulieu, E.E. (1997) Phosphorylation of the immunosuppressant FK506-binding protein FKBP52 by casein kinase II: regulation of HSP90-binding activity of FKBP52. *Proceedings of the National Academy of Sciences of the United States of America.* **94**, 14500–14505 [CrossRef PubMed](#)
- 196 Zhong, L., Qing, K., Si, Y., Chen, L., Tan, M. and Srivastava, A. (2004) Heat-shock treatment-mediated increase in transduction by recombinant adeno-associated virus 2 vectors is independent of the cellular heat-shock protein 90. *The Journal of biological chemistry.* **279**, 12714–12723 [CrossRef PubMed](#)
- 197 Zhao, W., Zhong, L., Wu, J., Chen, L., Qing, K., Weigel-Kelley, K.A., Larsen, S.H., Shou, W., Warrington, K.H., Jr. and Srivastava, A. (2006) Role of cellular FKBP52 protein in intracellular trafficking of recombinant adeno-associated virus 2 vectors. *Virology.* **353**, 283–293 [CrossRef PubMed](#)
- 198 Qing, K., Hansen, J., Weigel-Kelley, K.A., Tan, M., Zhou, S. and Srivastava, A. (2001) Adeno-associated virus type 2-mediated gene transfer: role of cellular FKBP52 protein in transgene expression. *Journal of virology.* **75**, 8968–8976 [CrossRef PubMed](#)
- 199 Dunn, D.M., Woodford, M.R., Truman, A.W., Jensen, S.M., Schulman, J., Caza, T., Remillard, T.C., Loiselle, D., Wolfgeher, D., Blagg, B.S., Franco, L., Haystead, T.A., Daturpalli, S., Mayer, M.P., Trepel, J.B., Morgan, R.M., Prodromou, C., Kron, S.J., Panaretou, B., Stetler-Stevenson, W.G., Landas, S.K., Neckers, L., Bratslavsky, G., Bourboulia, D. and Mollapour, M. (2015) c-Abi Mediated Tyrosine Phosphorylation of Aha1 Activates Its Co-chaperone Function in Cancer Cells. *Cell reports.* **12**, 1006–1018 [CrossRef PubMed](#)

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